DISSERTATION

on

STUDY OF LEPTOSPIROSIS

IN ADULT PATIENTS PRESENTING WITH FEVER AND JAUNDICE

M.D. DEGREE EXAMINATION

BRANCH I

(GENERAL MEDICINE)

THANJAVUR MEDICAL COLLEGE

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI

SEPTEMBER 2006

CERTIFICATE

This is to certify that this Dissertation entitled, "**STUDY OF LEPTOSPIROSIS IN ADULT PATIENTS PRESENTING WITH FEVER AND JAUNDICE**" is the bonafide record of work done by **Dr. G. VIKRAMRAJ**, submitted as partial fulfillment for the requirements of M.D. Degree Examinations Branch I, General Medicine, September 2006.

s/d - **Dr. V. BHASKARAN, M.D.,**
Unit Chief.

s/d - **Dr. K. GANDHI, M.D.,**
Head of the Department of Medicine,
Thanjavur Medical College Hospital,
Thanjavur.

s/d - **THE DEAN,**
Thanjavur Medical College,
Thanjavur.

ACKNOWLEDGEMENT

I am extremely thankful to our beloved Dean, Thanjavur Medical College, **Dr. S. BALAKRISHNAN, M.D.,** for granting me permission to undergo this study in Thanjavur medical college Hospital. I also thank our former Dean, DR. K .KALAISELVI, M.D.

I am very grateful to our Professor and Head of the Department of Medicine, **Dr. K.GANDHI M.D.**, for his moral support, philosophic guidance and constant help to undergo this study.

I am deeply indebted to my unit chief **Dr. V. BHASKARAN**, **M.D**., for his constant help and guidance to complete this study.

I am deeply indebted to the Chief of Nephrology, **Dr. N. MOHANDAS, M.D., D.M(Nephro),** for his invaluable guidance, perspectives, assortment of emerging views, constructive criticisms throughout the period of study.

I also thank my former unit chiefs **Dr. DHANASEELAN JEYAKUMAR, M.D**. and **Dr. N. JEEVA M.D.,** for their help and guidance.

I recall with gratitude the other unit Chiefs of Department of Medicine, **Dr. RAMASAMY, M.D.,** and **Dr. GOPALDAS, M.D.,** for having permitted me to work on their patients in their respective units.

I am extremely thankful to **Dr. C. PARANTHAGAN, M.D., Dr. BALASANJEEVI, M.D., Dr. C. GANESAN, M.D.,** and other Assistant Professors of Medicine for their guidance throughout the study.

I thank, Professor and Staff of the Department of Biochemistry for the guidance and permission to utilize their services.

I thank the Staff of Institute of Vector Control and Zoonoses (**IVCZ**), Hosur for their services in conducting serology for Leptospirosis.

I also thank our patients without whom the study would not have been possible.

I owe my thanks to almighty for successful completion of the study.

CONTENTS

INTRODUCTION

Leptospirosis is a worldwide zoonotic infection with a much greater incidence in tropical regions and has now been identified as one of the emerging infectious diseases. The epidemiology of leptospirosis has been modified by changes in animal husbandry, climate, and human behavior. Resurgent interest in leptospirosis has resulted from large outbreaks that have received significant publicity. Awareness has increased internationally over the past decade that leptospirosis is a major globally important public health threat, both in developing countries and industrialized countries. The economic burden imposed by this disease is unknown, yet it is widely recognized that the incidence of leptospirosis is remarkably underestimated and the disease under diagnosed in endemic regions. Leptospirosis is estimated to affect tens of millions of humans annually with case fatality rates ranging from 5 to 25%. Case finding and reporting have been limited, nonsustained and biased.

This study of incidence and clinical profile of leptospirosis Among patients presenting with fever and jaundice was conducted in Thanjavur Medical College Hospital, Thanjavur, sofar a non-endemic region for Leptospirosis. Though cases of Leptospirosis occur sporadically in this hospital a study about the clinical profile is essential in epidemiological aspect.

AIM OF THIS STUDY

1. To study the incidence of Leptospirosis among unexplained fever and jaundice cases admitted in TMCH.
2. To analyze the relevant epidemiological data like seasonal and demographic clustering of cases.
3. To analyze the occupation of seropositive cases.
4. To analyze the clinical features among the positive cases confirmed by serology.
5. To analyze the Investigations done on seropositive cases.
6. To study the incidence of complications.
7. To analyze about the outcome among positive cases.

REVIEW OF LITERATURE

HISTORY:

Leptospirosis was certainly recognized as an occupational hazard of rice harvesting in ancient China ,and the Japanese name *akiyami*, or autumn fever, persists in modern medicine[1]. With hindsight, clear descriptions of leptospiral jaundice can be recognized as having appeared earlier in the 19th century, some years before the description by Weil in 1886. The etiology of leptospirosis was demonstrated independently in 1915 in Japan and Germany. In Japan, Inada and Ido detected both spirochetes and specific antibodies in the blood of Japanese miners with infectious jaundice, and two groups of German physicians studied German soldiers afflicted by "French disease" in the trenches of northeast France. Uhlenhuth and Fromme and Hubener and Reiter, detected spirochetes in the blood of guinea pigs inoculated with the blood of infected soldiers[1].

The importance of occupation as a risk factor was recognized early. The role of the rat as a source of human infection was discovered in 1917. while the potential for leptospiral disease in dogs was recognized, but clear distinction between canine infection with *L. interrogans* serovars icterohaemorrhagiae and canicola took several years. Leptospirosis in livestock was recognized some years later[8].

CAUSATIVE ORGANISM:

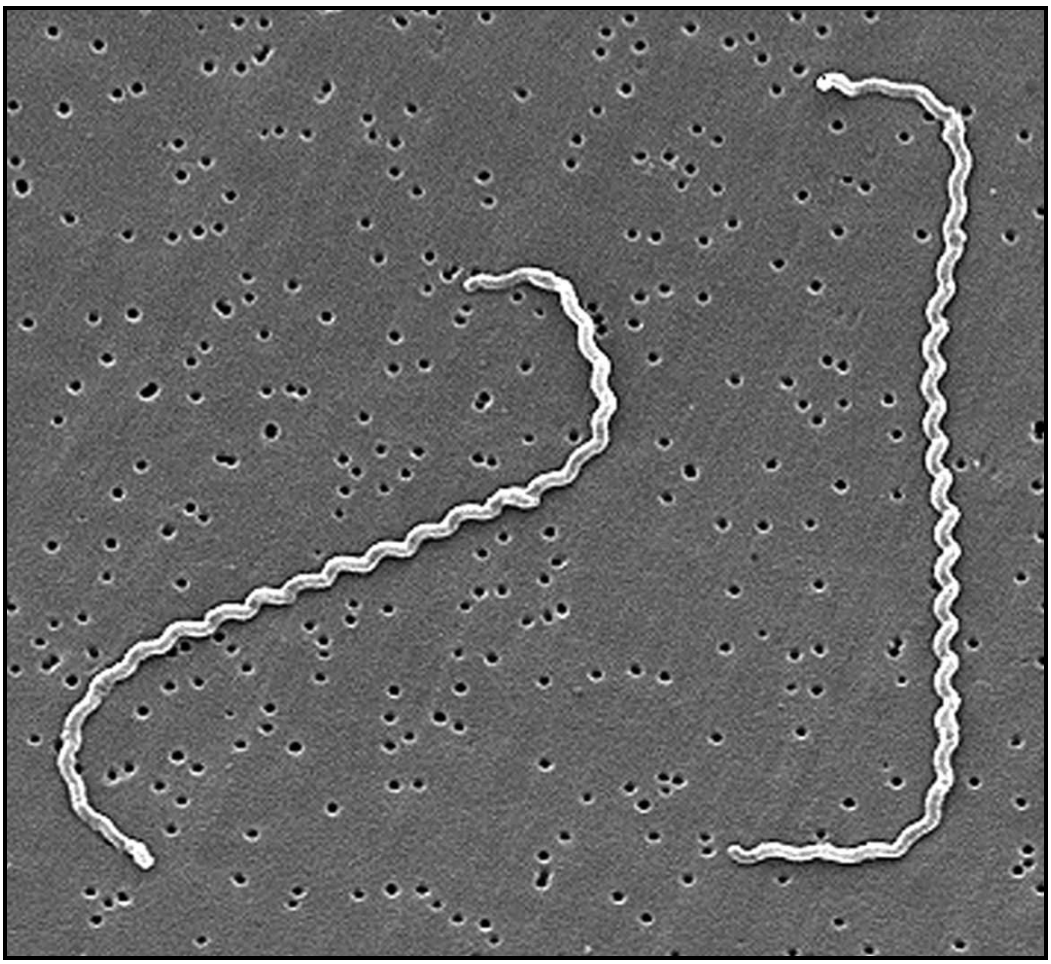
Biology of Leptospires:

Leptospires are tightly coiled spirochetes, usually 0.1 µm by 6 to 0.1 by 20 µm, (*leptos*- meaning fine or thin). The cells have pointed ends, either or both of which are usually bent into a distinctive hook .Two axial filaments (periplasmic flagella) with polar insertions are located in the periplasmic space. Leptospires exhibit two distinct forms of movement, translational and nontranslational. Leptospiral lipopolysaccharide has a composition similar to that of other gram-negative bacteria ,but has lower endotoxic activity. Leptospires are obligate aerobes with an optimum growth temperature of 28 to 30°C. They produce both catalase and oxidase . They grow in simple media enriched with vitamins (vitamins B_2 and B_{12} are growth factors), long-chain fatty acids, and ammonium salts . Long-chain fatty acids are utilized as the sole carbon source and are metabolized by β-oxidation[1,2,50].

Culture Methods:

Growth of leptospires in media containing either serum or albumin plus polysorbate and in protein-free synthetic media has been described. Several liquid media containing rabbit serum were described by Fletcher, Korthoff, Noguchi, and Stuart. The most widely used medium in current practice is based on the oleic acid-albumin medium EMJH. It contains Tween 80 and bovine serum albumin[1,50].

Scanning electron micrograph of *L. interrogans* serovar icterohaemorrhagiae strain bound to a 0.2-µm membrane filter[1,2]



CLASSIFICATION

Serological classification: Prior to 1989, the genus *Leptospira* was divided into two species, *L. interrogans*, comprising all pathogenic strains, and *L. biflexa*, containing the saprophytic strains isolated from the environment. *L. biflexa* was differentiated from *L. interrogans* by the growth of the former at 13°C and growth in the presence of 8-azaguanine (225 µg/ml) and by the failure of *L. biflexa* to form spherical cells in 1 M NaCl.

Both *L. interrogans* and *L. biflexa* are divided into numerous serovars defined by agglutination after cross-absorption with homologous antigen . If more than 10% of the homologous titer remains in at least one of the two antisera on repeated testing, two strains are said to belong to different serovars . Over 60 serovars of *L. biflexa* have been recorded . Within the species *L. interrogans* over 200 serovars are recognized; additional serovars have been isolated but have yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups . While serogroups have no taxonomic standing, they have proved useful for epidemiological understanding.

Genotypic classification: The phenotypic classification of leptospires has been replaced by a genotypic one, in which a number of genomospecies include all serovars of both *L. interrogans* and *L. biflexa*. Genetic

heterogeneity was demonstrated some time ago, and DNA hybridization studies led to the definition of 10 genomospecies of *Leptospira* . An additional genomospecies, *L. kirschneri*, was added later. After an extensive study of several hundred strains, workers at the Centers for Disease Control (CDC) more recently defined 16 genomospecies of *Leptospira* that included those described previously and adding five new genomospecies , one of which was named *L. alexanderi*. An additional species, *L. fainei*, has since been described, which contains a new serovar, hurstbridge . The genotypic classification of leptospires is supported by multilocus enzyme electrophoresis data ,but recent studies suggest that further taxonomic revisions are likely .The genomospecies of *Leptospira* do not correspond to the previous two species (*L. interrogans* and *L. biflexa*), and indeed, pathogenic and nonpathogenic serovars occur within the same species. Thus, neither serogroup nor serovar reliably predicts the species of *Leptospira* . Moreover, recent studies have included multiple strains of some serovars and demonstrated genetic heterogeneity within serovars. In addition, the phenotypic characteristics formerly used to differentiate *L. interrogans* sensu lato from *L. biflexa* sensu lato do not differentiate the genomospecies.

The reclassification of leptospires on genotypic grounds is taxonomically correct and provides a strong foundation for future classifications.

Species of *Leptospira* and some Pathogenic Serovars[2]

Species	Selected Pathogenic Serovars
L. interrogans	Icterohaemorrhagiae, Copenhageni, Canicola, Pomana, Australis, Autumnalis, Pyrogenes, Bratislava, Lai.
L. noguchii	Panama, Pomona.
L. borgpetersenii	Ballum, Hardjo, Javanica.
L. santarosai	Bataviae.
L. kirschneri	Bim, Bulgarica, Grippotyphosa, Cynopteri.
L. weilii	Celledoni, Sarmin.
L. alexanderi	Manhao 3.
***Leptospira* genomspecies 1**	Sichuan.
L. fainei	Hurstbridge.
L. Meyeri	Sofia
L. Inadai	Indeterminate
L. wolbachii	Non-pathogens
L. biflexa	Non-pathogens
***Leptospira* genomspecies 3**	Non-pathogens
***Leptospira* genomspecies 4**	Non-pathogens
***Leptospira* genomspecies 5**	Non-pathogens
L. parva	Non-pathogens

EPIDEMIOLOGY:

Leptospirosis is presumed to be the most widespread zoonosis in the world[1] . The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal. The incidence is significantly higher in warm-climate countries than in temperate regions; this is due mainly to longer survival of leptospires in the environment in warm, humid conditions. However, most tropical countries are also developing countries, and there are greater opportunities for exposure of the human population to infected animals, whether livestock, domestic pets, or wild or feral animals. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospires, and during rainy seasons in warm-climate regions, where rapid dessication would otherwise prevent survival.

The usual portal of entry is through abrasions or cuts in the skin or via the conjunctiva; infection may take place via intact skin after prolonged immersion in water, but this usually occurs when abrasions are likely to occur and is thus difficult to substantiate. Water-borne transmission has been documented; point contamination of water supplies has resulted in several outbreaks of leptospirosis . Inhalation of water or aerosols also may result in infection via the mucous membranes of the respiratory tract. Rarely, infection may follow animal bites. Direct transmission between humans has been

demonstrated rarely. However, excretion of leptospires in human urine months after recovery has been recorded. It is thought that the low pH of human urine limits survival of leptospires after excretion. Transmission by sexual intercourse during convalescence has been reported[1].

Animals, including humans, can be divided into maintenance hosts and accidental (incidental) hosts. The disease is maintained in nature by chronic infection of the renal tubules of maintenance hosts . A maintenance host is defined as a species in which infection is endemic and is usually transferred from animal to animal by direct contact. Infection is usually acquired at an early age, and the prevalence of chronic excretion in the urine increases with the age of the animal. Other animals (such as humans) may become infected by indirect contact with the maintenance host. Animals may be maintenance hosts of some serovars but incidental hosts of others, infection with which may cause severe or fatal disease. The most important maintenance hosts are small mammals, which may transfer infection to domestic farm animals, dogs, and humans. The extent to which infection is transmitted depends on many factors, including climate, population density, and the degree of contact between maintenance and accidental hosts. Different rodent species may be reservoirs of distinct serovars, but rats are generally maintenance hosts for serovars of the serogroups lcterohaemorrhagiae and Ballum, and mice are the maintenance hosts for serogroup Ballum. Domestic

animals are also maintenance hosts; dairy cattle may harbor serovars hardjo, pomona, and grippotyphosa; pigs may harbor pomona, tarassovi, or bratislava; sheep may harbor hardjo and pomona; and dogs may harbor canicola

Human infections may be acquired through occupational, recreational, or vocational exposures. Occupation is a significant risk factor for humans. Direct contact with infected animals accounts for most infections in farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers, and other occupations which require contact with animals. Indirect contact is important for sewer workers, miners, soldiers, septic tank cleaners, fish farmers, gamekeepers, canal workers, rice field workers, taro farmers, banana farmers, and sugar cane cutters.[1, 44, 55]

Survival of pathogenic leptospires in the environment is dependent on several factors, including pH, temperature, and the presence of inhibitory compounds. Most studies have used single serovars and quite different methodologies, but some broad conclusions may be drawn. Under laboratory conditions, leptospires in water at room temperature remain viable for several months at pH 7.2 to 8.0, but in river water survival is shorter and is prolonged at lower temperatures. The presence of domestic sewage decreases the survival time to a matter of hours, but in an oxidation ditch filled with cattle slurry, viable leptospires were detected for several weeks

Important Leptospiral Infections[50]

Serotype	*Disease*	*Clinical Picture*	*Animal Reservoir*	*Distribution*
Icterohemorrhagiae	***Weils Disease***	Fever, jaundice, hemorrhages	Rat	Worldwide
Canicola	***Canicola fever***	Influenza like, aseptic meningitis	Dog	Worldwide
Grippotyphosa	***Swamp or marsh fever***	Fever, prostration, aseptic meningitis	Field mice	Europe. Africa, SE Asia, USA
Pomona	***Swineherd's disease***	Fever	Pig	America, Europe, Middle East, Indonesia, Australia
Hebdomadis	***Seven day fever***	Fever, Lymphadenopathy	Field mice	Japan, Europe, USA
Fortbragg	***Pretibial fever Fortbragg fever***	Fever, rash over tibia	Not known	Japan, SE Asia, USA
Pyrogenes	***Febrile Spirochetosis***	Fever	Pig	SE Asia, Europe, USA
Bataviae	***Indonesian Weil's disease***	Fever	Rat	SE Asia, Africa, Europe
Hardjo	***Dairy farm fever***	Fever	Cattle	UK, USA, New Zealand

PATHOLOGY AND PATHOGENESIS.

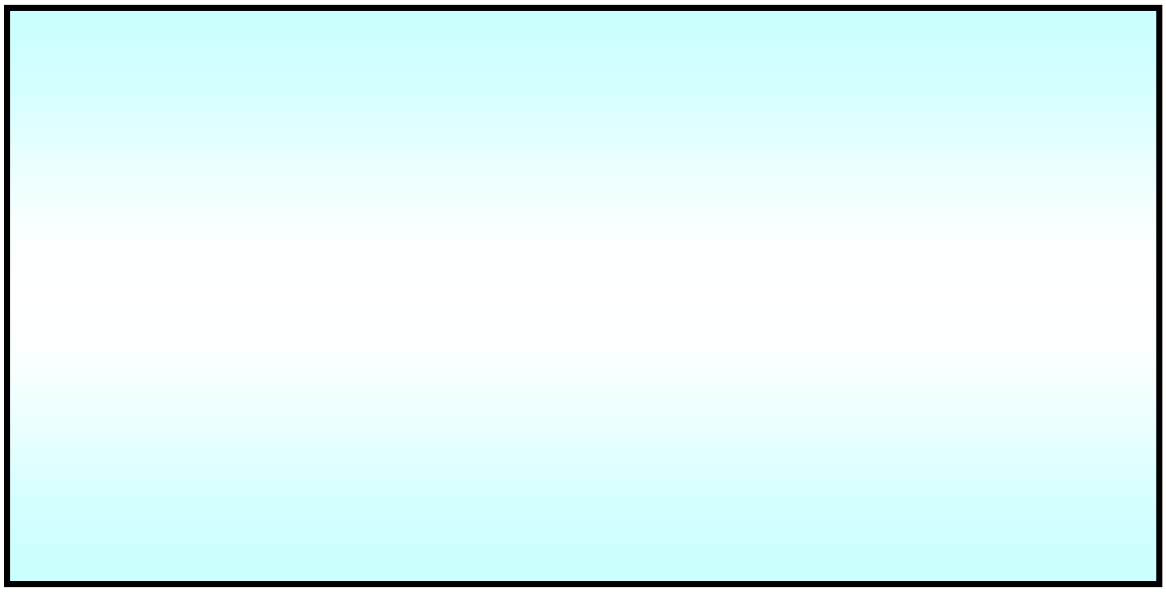
Leptospira penetrate intact mucous membranes and abraded skin and disseminate widely via the bloodstream. Leptospirosis is an infectious vasculitis, with damage to capillary endothelial cells responsible for the major clinical manifestations of disease, including renal tubular and hepatic dysfunction, myocarditis, and pulmonary hemorrhage. Intra- to extra vascular fluid shifts secondary to endothelial damage lead to hypovolemia, which complicates renal dysfunction and can lead to shock. Fatal cases are

associated with widespread hemorrhage of mucosal, skin, and serosal surfaces.

In **LIVER**, the damage is at the subcellular level. An endotoxin-like substance in the wall of Spirochetes has been suggested. Plasma TNF-α levels have been related to severity of organ involvement. Liver necrosis is minimal and focal. Zone 3 necrosis is absent. Active hepatocellular regeneration is out of proportion to the cell damage. Swollen Kupfer cells have Leptospiral debris. Leucocyte infiltration and bile thrombi are prominent in deeply jaundiced. Cirrhosis is not a sequela.[53]

Examination of the **Kidneys** from autopsies[23] has revealed ischemic damage, including epithelial cell necrosis in the distal convoluted tubules and the ascending loop of Henle, and interstitial nephritis but only rarely glomerular damage. **Muscle** biopsies have demonstrated focal necrotic changes with a mild mononuclear infiltrate. Only rarely is the spirochete visualized in the infected tissue. Hemorrhagic myocarditis[2,42] has been observed frequently in autopsies. The secondary "immune" phase of leptospirosis is associated with the clearance of the organism from blood and CSF and the appearance of agglutinating anti-Leptospira antibodies.

Possible Determinants of Leptospiral Severity: [54]



CLINICAL FEATURES:

Symptoms develop 7 to 12 days after exposure. The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase and thus occur during the second week of the illness[1].

Anicteric Leptospirosis:

The great majority of infections caused by leptospires are either sub clinical or of very mild severity, and patients will probably not seek medical attention. A smaller proportion of infections, but the overwhelming majority of the recognized cases, present with a febrile illness of sudden onset.

Other symptoms include chills, headache, myalgia, abdominal pain, conjunctival suffusion, and less often a skin rash. If present, the rash is often transient, lasting less than 24 h. The fever may be biphasic and may recur after a remission of 3 to 4 days. The headache is often severe, resembling that occurring in dengue, with retro-orbital pain and photophobia. Myalgia affecting the lower back, thighs, and calves is often intense. This anicteric syndrome usually lasts for about a week, and its resolution coincides with the appearance of antibodies.

Icteric Leptospirosis

Icteric leptospirosis is a much more severe disease in which the clinical course is often very rapidly progressive. Between 5 and 10% of all patients with leptospirosis have the icteric form of the disease. Severe cases often present late in the course of the disease, and this contributes to the high mortality rate, which ranges between 5 and 15%. The jaundice occurring in leptospirosis is not associated with hepatocellular necrosis, and liver function returns to normal after recovery. Serum bilirubin levels may be high, and many weeks may be required for normalization. There are moderate rises in transaminase levels, and minor elevation of the alkaline phosphatase level usually occurs.

The important Differential Diagnosis includes severe falciparum malaria, enteric fever, viral hepatitis and dengue hemorrhagic fever. In India, the seasonal pattern of leptospirosis coincides with that of malaria. Rickettsia tsutsugamushi infection and Hanta virus infection produce clinical a illness identical to severe leptospirosis. Serology differentiates the illnesses.[7]

Difference between Weil's Disease and Viral Hepatitis: [53]

	Weils Disease	Viral Hepatitis
Onset	Sudden	Gradual
Headache	Constant	Occasional
Muscle Pain	Severe	Mild
Conjunctival Suffusion	Present	Absent
Prostration	Great	Mild
Disorientation	Common	Rare
Bleeding diathesis	Common	Rare
Nausea/Vomiting	Present	Present
Abdominal Discomfort	Common	Common
Bronchitis	Common	Rare
Albuminuria	Present	Absent
Leucocyte count	Polymorph leucocytosis	Leucopenia with lymphocytosis
Creatine Phospho kinase	Elevated	Usually normal

COMPLICATIONS

The complications of severe leptospirosis emphasize the multisystemic nature of the disease. **Renal** involvement is common in leptospirosis.[14,15,16] Bacterial invasion, inflammatory process, haemodynamic alterations and direct toxicity of bacterial products are thought to be responsible for the development of nephropathy. Pathologically, all renal structures are involved. Interstitial nephritis is the basic lesion, and is observed even in patients without clinical renal manifestations. Tubular necrosis is the important pathological counterpart of acute renal failure. The clinical spectrum of renal manifestations includes mild urinary sediment change, hypokalemia, tubular dysfunction, decreased response to fluid load and acute renal failure (ARF) [22]. Leptospirosis is a common cause of acute renal failure (ARF), which occurs in 16 to 40% of cases. A distinction may be made between patients with prerenal azotemia (non-ARF) and those with ARF. Patients with prerenal azotaemia may respond to rehydration, and decisions regarding dialysis can be delayed for up to 72 h. ARF reflects the severity of leptospirosis, is catabolic and is commonly associated with cholestatic jaundice. Severe renal failure may be complicated by multiple organ involvement. [22]

Renal failure with hyperbilirubinemia represents a severe form of renal dysfunction with oligo-anuria and prolonged clinical course. Mild renal failure is usually anicteric and non-oliguric and without complication. Besides antibiotic treatment, early and frequent dialysis is life saving. ARF with major organ failure has unfavorable outcome.

Pulmonary hemorrhage may be severe enough to cause death. Patients may present with a spectrum of symptoms, ranging from cough, dyspnea, and hemoptysis to adult respiratory distress syndrome. Radiography generally reveals diffuse small opacities which may be widely disseminated or which may coalesce into larger areas of consolidation, with increasing severity of symptoms. Pleural effusions may occur.[2,24,36.]

Clinical evidence of myocardial involvement, including abnormal T waves may be seen.[2,24,42].

Ocular Involvement:

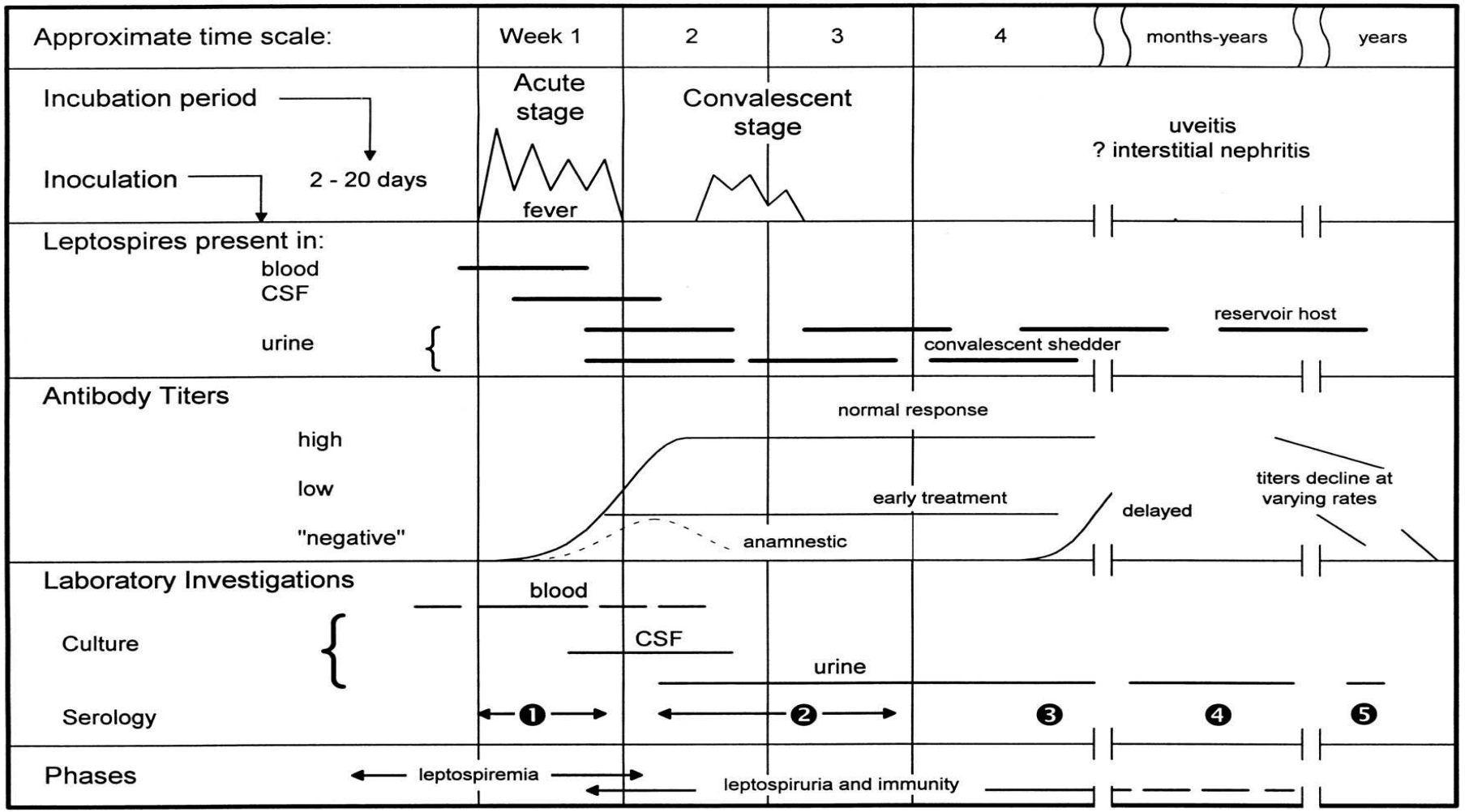
Conjunctival suffusion is seen in the majority of patients in some series. Conjunctival suffusion in the presence of scleral icterus is said to be pathognomonic of Weil's disease. Anterior uveitis, either unilateral or bilateral, occurs after recovery from the acute illness in a minority of cases. Uveitis may present weeks, months, or occasionally years after the acute stage. Chronic visual disturbance, persisting 20 years or more after the acute illness, has been reported.[38]

Hypopyon and Cataract in Leptospiral uveitis[38]

Other Complications

Acute infection in pregnancy has been reported to cause abortion and fetal death. In one of the cases reported by Chung et al., leptospires were isolated from amniotic fluid, placenta, and cord blood; the infant was mildly ill and was discharged at 2 weeks of age. In another case, a neonate developed jaundice and died 2 days after birth.[2,24]

Rare complications include cerebrovascular accidents, rhabdomyolysis, thrombotic thrombocytopenic purpura, acute acalculous cholecystitis, erythema nodosum, aortic stenosis, Kawasaki syndrome, reactive arthritis, epididymitis, nerve palsy, male hypogonadism, and Guillain-Barré syndrome. Cerebral arteritis, resembling Moyamoya disease, has been reported in a series of patients from China.[24]



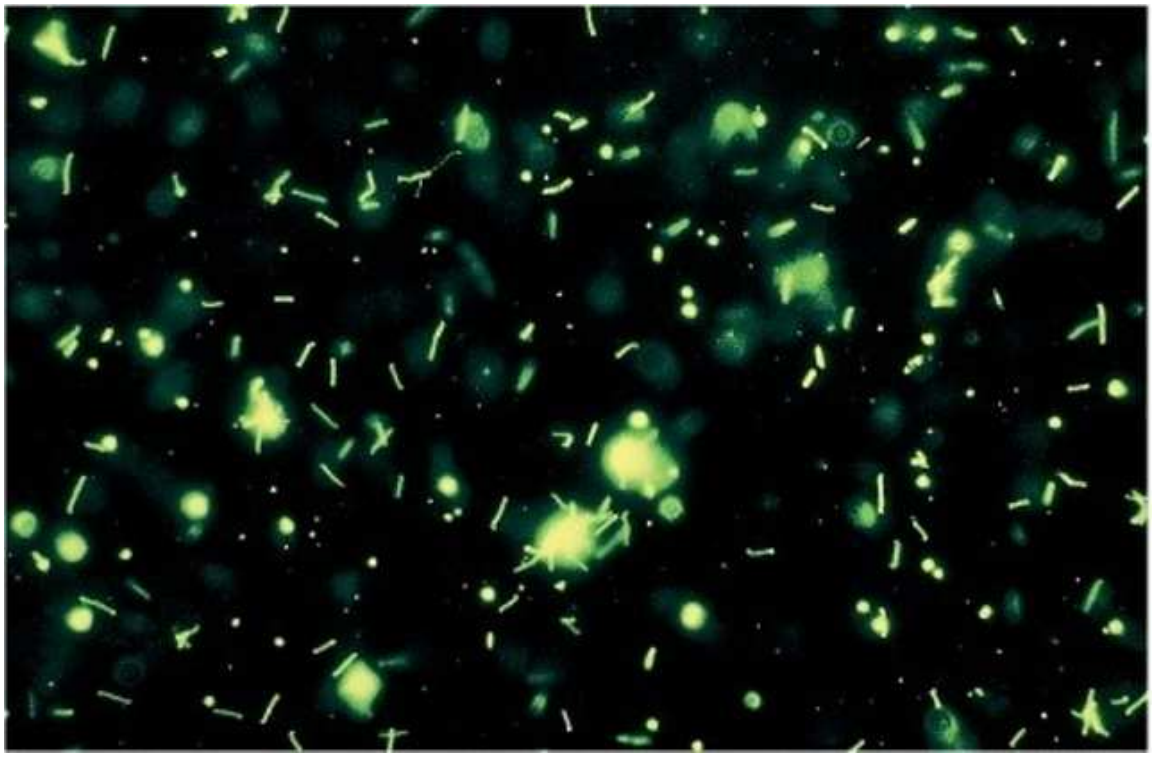
Biphasic nature of leptospirosis and relevant investigations at different stages of disease. Specimens 1 and 2 for serology are acute-phase specimens, 3 is a convalescent-phase sample which may facilitate detection of a delayed immune response, and 4 and 5 are follow-up samples which can provide epidemiological information, such as the presumptive infecting serogroup. [1,2,3]

DIAGNOSIS

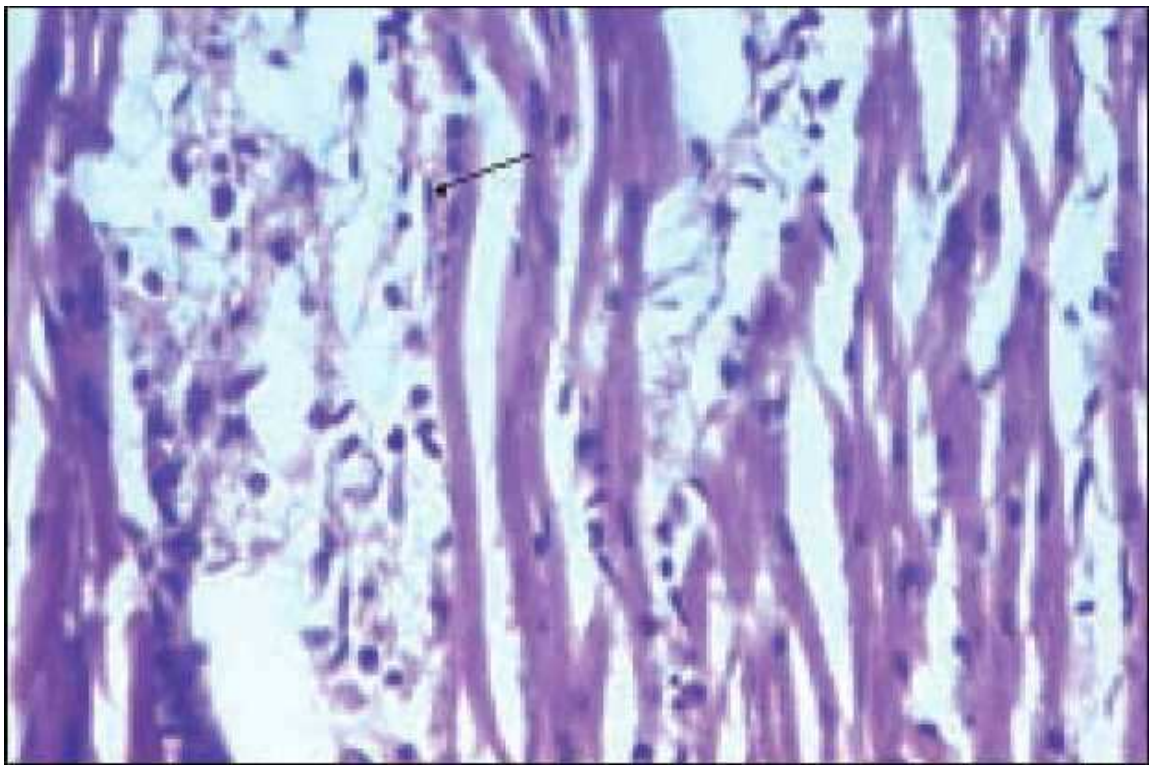
Microscopic Demonstration

Leptospires may be visualized in clinical material by dark-field microscopy or by immunofluorescence or light microscopy after appropriate staining[50]. Approximately 10 leptospires/ml are necessary for one cell per field to be visible by dark-ground microscopy (DGM). A quantitative buffy coat method has been shown to have a sensitivity of approximately 10 leptospires/ml. Microscopy of blood is of value only during the first few days of the acute illness, while leptospiremia occurs[1]. Dark-field microscopic examination of body fluids such as blood, urine, CSF, and dialysate fluid has been used but is both insensitive and lacks specificity. The drawbacks of DGM on clinical specimens as a diagnostic tool has been that both false positive and false negative diagnosis can be easily made even in experienced hands.

Staining methods have been applied to increase the sensitivity of direct microscopic examination. These include immunofluorescence staining of bovine urine, water, and soil and immunoperoxidase staining of blood and urine. A variety of histopathological stains have been applied to the detection of leptospires in tissues. Leptospires were first visualized by silver staining, and the Warthin-Starry stain is widely used for histologic examination. Recently, immunohistochemical methods have been applied.

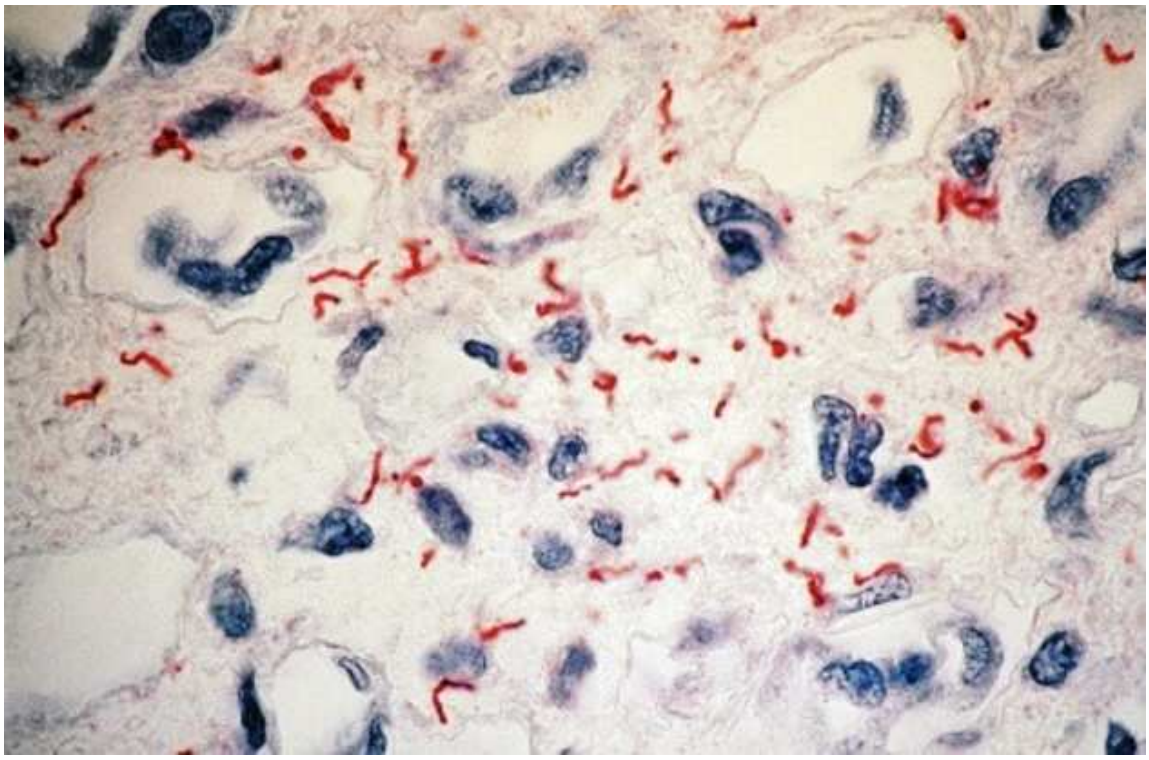


Leptospires viewed by dark-field microscopy
(original magnification × 100).



Section from liver showing mononuclear inflammation in portal tracts

Kidney sections stained by **silver staining** showing presence of multiple leptospires in tubules.[2]



Kidney sections stained by **immunohistochemical staining** showing presence of multiple leptospires in tubules. [2]

Culture

Isolation of Leptospires:

Leptospiremia occurs during the first stage of the disease, beginning before the onset of symptoms, and ends by the first week of the illness. Therefore blood cultures should be taken as soon as possible after the patient's presentation. One or two drops of blood are inoculated into 10 ml of semisolid medium containing 5-fluorouracil at the patient's bedside. For the greatest recovery rate, multiple cultures should be performed, but this is rarely possible. Inoculation of media with dilutions of blood samples may increase recovery. Rapid detection of leptospires by radiometric methods has been described. Leptospires survive in conventional blood culture media for a number of days. Rarely, leptospires have been isolated from blood weeks after the onset of symptoms. Isolation of leptospires from clinical samples gives a definitive diagnosis and also aids in identifying the prevalent serovar[1,20].

Apart from blood, CSF and dialysate fluid can also be cultured during the first week of illness. Urine can be cultured from the beginning of the second week of symptomatic illness. The duration of urinary excretion varies but may last for several weeks. Survival of leptospires in voided human urine is limited, so urine should be immediately centrifuged, followed by resuspending the sediment in phosphate-buffered saline (to neutralize the pH) and inoculating into semisolid medium containing 5-fluorouracil.

Cultures are incubated at 28 to 30°C and examined weekly by dark-field microscopy for up to 13 weeks before being discarded. Contaminated cultures may be passed through a 0.2-µm or 0.45-µm filter before subculture into fresh medium.

Though the use of culture confirms diagnosis, it is rarely used, as it is very tedious, complicated, expensive, technically demanding, time consuming, requiring prolonged incubation (minimum 1 month before declaring a sample negative) and may not be successful (low sensitivity). The organism also has a relatively long doubling time (6 to 8 h or more). Additionally they are highly infectious organisms requiring 'Biosafety level II' facilities.

Susceptibility testing:

Leptospires are susceptible to beta-lactams, macrolides, tetracyclines, fluoroquinolones and streptomycin. Problems in the determination of susceptibility include the long incubation time required, the use of media containing serum and the difficulty in quantifying growth accurately. These constraints have limited the development of rapid, standardized methods for susceptibility testing.

Antigen detection

Detection of leptospiral antigens in clinical material offer greater specificity than DGM while having the potential for greater sensitivity. Radioimmunoassay (RIA) can detect 10^4sub to 10^5sub leptospires/ml and an enzyme-linked immunosorbent assay (ELISA) method can detect 10^5sub leptospires/ml. A chemiluminescent immunoassay (CLIA) has been applied to human blood and urine but has been less sensitive than earlier ELISA. Recently, immunomagnetic antigen capture has been combined with fluoroimmunoassay to detect as few as 10^2sub leptospires/ml in urine of cattle infected with serovar *hardjo* .

Genus-specific serological tests for diagnosis of Leptospirosis[1]

- **Complement fixation test**
- **Sensitized erythrocyte lysis**
- **Macroscopic slide agglutination**
- **Immunfluorescence**
- **Patoc slide agglutination test**
- **Indirect hemagglutination**
- **Counterimmunoelectrophoresis**
- **ELISA**
- **Microcapsule agglutination**
- **Dot-ELISA**
- **IgM dipstick**
- **Latex agglutination**

Antibody detection

Most cases of leptospirosis are diagnosed by serology. Antibodies can become detectable by the 6th to 10th day of disease and generally reach peak levels within 3 to 4 weeks. Antibody levels then gradually recede but may remain detectable for years. Serological methods can be divided into two groups: those, which are genus specific and those which are serogroup specific. The definitive serological investigation in leptospirosis remains the microscopic agglutination test (MAT).

Microscopic agglutination test (MAT):

The reference method for serological diagnosis of leptospirosis is the MAT, in which patient sera are reacted with live antigen suspensions of leptospiral serovars[1]. After incubation, the serum-antigen mixtures are examined microscopically for agglutination and the titers are determined. Formerly, the method was known as the agglutination-lysis test because of the formation of lysis balls or lysis globules of cellular debris in the presence of high-titered antiserum. However, these are tightly agglutinated clumps of leptospires containing live cells and not debris. Several modifications of earlier methods led to a MAT method, which can be performed and read in microtiter trays. The MAT is read by dark-field microscopy. The end point is the highest dilution of serum at which 50% agglutination occurs. Because of the difficulty in detecting when 50% of the leptospires are agglutinated, the

end point is determined by the presence of approximately 50% free, unagglutinated leptospires compared to the control suspension Considerable effort is required to reduce the subjective effect of observer variation, even within laboratories[20]. Different laboratories use different cut-off titres ranging from 1 in 100 to 1 in 800 for diagnosis and may result in overdiagnosis and overestimation of disease burden. The importance of determination of base line titres in the community hence cannot be overemphasised.

Interpretation of this test is complicated by the high degree of cross-reaction that occurs between different serogroups, especially in acute-phase samples. This is to some extent predictable, and patients often have similar titers to all serovars of an individual serogroup. Of note, "paradoxical" reactions in which the highest titers are detected to a serogroup unrelated to the infecting one, are also common. Thus MAT is a complex test to control, perform, and interpret. Live cultures of all serovars required for use as antigens need to be maintained. Moreover, the repeated weekly subculture of large numbers of strains presents hazards for laboratory workers[20].

Paired sera are required to confirm a diagnosis with certainty. A fourfold or greater rise in titer between paired sera confirms the diagnosis regardless of the interval between samples[2,3,5,6]. The interval between the first and second samples greatly depends on the delay between onset of symptoms and presentation of the patient. If symptoms of overt leptospirosis are present,

an interval of 3 to 5 days may be adequate to detect rising titers. However, if the patient presents earlier in the course of the disease or if the date of onset is not known precisely, then an interval of 10 to 14 days between samples is more appropriate[1,20].

In the Current **CDC** Case Definition, a titer of ≥ 200 is used to define a probable case with a clinically compatible illness.[1,4] In areas where leptospirosis is endemic, a single titer of ≥800 in symptomatic patients is indicative of leptospirosis. The National Institute of Communicable Diseases (**NICD**), New Delhi Case Definition also advocates a MAT titre of >200.[5]

Other Serological Tests

Because of the complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infection have been developed. Complement fixation (CF) was widely used but methods were not standardized. CF tests have generally been replaced by ELISA methods.

IgM antibodies become detectable during the first week of illness allowing the diagnosis to be confirmed and treatment initiated while it is likely to be most effective. Antibody levels are generally low or absent during very early infection. IgM detection has repeatedly been shown to be more sensitive than MAT when the first specimen is taken early in the acute phase of the illness. However most of the commercially available ELISA kits use non-pathogenic *L.biflexa patoc 1* strain as an antigen. The drawback of this

test is that the infective serovar cannot be assessed. Though the test is more sensitive than MAT it is less specific.

An IgM-specific dot-ELISA has been developed in which polyvalent leptospiral antigen was dotted onto nitrocellulose filter disks in microtiter tray wells, allowing the use of smaller volumes of reagents. Further modifications of this approach have been used to detect IgG and IgA, in addition to IgM and have employed an immunodominant antigen and a polyester fabric-resin support in place of nitrocellulose.

A commercial slide agglutination assay has been recently found to be as sensitive and specific as an IgM-ELISA, while remaining reactive for a shorter time after recovery than either the IgM-ELISA, or the MAT. A number of methods using sensitized red blood cells have been described. The extraction of an erythrocyte-sensitizing substance led to the development of both a hemolytic assay requiring complement and a hemagglutination assay, and a number of modifications of the latter have been reported. These assays detect both IgM and IgG antibodies. The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population.

The indirect hemagglutination assay (IHA) developed at CDC was shown to have a sensitivity of 92% and specificity of 95% compared with the MAT.

A microcapsule agglutination test using a synthetic polymer in place of red

blood cells has been evaluated extensively. The microcapsule agglutination test is reportedly more sensitive than either the MAT or an IgM-ELISA in early acute phase samples, but failed to detect infections caused by some serovars. Advantages of this direct agglutination method is that it can be applied without modification to sera from other animal species.

Other techniques applied to the detection of leptospiral antibodies include immunofluorescence, RIA, counterimmunoelectrophoresis, and thin-layer immunoassay. These methods are not widely used.

Molecular Diagnosis:

Leptospiral DNA has been detected in clinical material by dot-blotting and in-situ hybridization. Several primer pairs for PCR detection of leptospires have been described. A limitation of PCR-based diagnosis of leptospirosis is the inability of most PCR assays to identify the infecting serovar.

Molecular typing

There has been great interest in molecular methods for identification and subtyping because of the difficulties associated with serological identification of leptospiral isolates,. Methods employed have included digestion of chromosomal DNA by restriction endonucleases (REA), restriction fragment length polymorphism (RFLP), ribotyping, pulsed field gel electrophoresis, and a number of PCR-based approaches.

TREATMENT

Antibiotic treatment is most beneficial if started within 4 days of illness; unfortunately, the diagnosis of leptospirosis is rarely made this rapidly.

Doxycycline, 100 mg orally twice a day for 7 days, started within 48 hours of illness, decreased the duration of illness by 2 days in one study; ***Penicillin*** at a dose of 2.4 to 3.6 million units per day also has been successful early treatment. A beneficial effect of antibiotic therapy later in disease course has not been uniformly seen. While a randomized, double-blinded trial of penicillin treatment (1.5 million units intravenously every 6 hours for 7 days) started on average nine days into illness showed a decrease in fever duration from 11.6 to 4.7 days and in elevated serum creatinine level from 8.3 to 2.7 days, a second randomized trial of penicillin in patients with icteric leptospirosis and a median duration of illness of 1 week demonstrated no beneficial effect. Jarish-Herxheimer reactions (fever, rigors, hypotension, and tachycardia) rarely occur upon initiation of antibiotic therapy. Supportive care and treatment of the hypotension, renal failure (including dialysis), and hemorrhage, which can complicate leptospirosis, are crucial for a good outcome.

Antimicrobial Agents Recommended for Treatment and Chemoprophylaxis of Leptospirosis [2,3,7]

Indication	Compound	Dosage
Chemoprophylaxis	**Doxycycline**	200mg PO orally once per week
Treatment of mild Leptospirosis#	**Doxycycline**	100 mg bid PO
	Ampicillin	500 – 750 mg q6h PO
	Amoxixillin	500 mg q6h PO
Treatment of moderate to severe Leptospirosis#	**Penicillin G**	1.5 MU IV q6h
	Ceftriaxone	1 g IV q24h
	Ampicillin	0.5 – 1 g IV q6h

All regimens used for the treatment are administered for 7 days

Immunization of animals is not necessarily effective at preventing human disease, since leptospiruria can still occur in immunized animals[1]. Because asymptomatically infected wild animals can chronically excrete large numbers of spirochetes in their urine, controlling environmental sources of leptospirosis is difficult if not impossible. Occupationally exposed individuals (abbatoir workers, veterinarians) should wear protective clothing to prevent exposure of skin and mucous membranes to potentially infected urine. Bodies of water associated with recreational exposures to leptospirosis may need to be placed off limits[1].

MATERIALS AND METHODS

Selection criteria:

- Patients with acute onset of fever and jaundice of less than 10 days duration were included in the study.
- All patients were ≥ 12 years of age
- All Pediatric cases were excluded from this study.
- Patients with chronic liver disease were excluded from this study.

No of patients selected: **164**

Period of study: Between 1st August 2004 to 31st December 2005

All patients were adult of ≥ 12 years of age who were admitted as in-patients in the Medicine Department in Thanjavur Medical College Hospital for acute onset of fever and jaundice.

Study design: Prospective study.

METHODS

All patients admitted in Thanjavur Medical College Hospital with fever and jaundice were evaluated. History was taken regarding duration of fever and duration of jaundice, occupation and history of travel.

Symptoms other than fever and jaundice *viz.* headache, nausea, vomiting, abdominal pain, diarrhea, cough, hemoptysis, anorexia, myalgia, conjunctival suffusion, oliguria, hematuria, etc., were noted.

Signs like rashes, signs of dehydration, lymphadenopathy, hepatomegaly, splenomegaly, anaemia, abdominal tenderness, muscle tenderness, signs of meningeal irritation, altered sensorium, etc., were also noted.

Investigations like routine urinary examination, urine for bile salts and bile pigments, complete hemogram, ESR, Liver function tests, Renal parameters like blood urea, serum creatinine, serum electrolytes and Creatine Phosphokinase levels were done on admission.

Blood was taken for Serology for Leptospirosis for all patients with history of more than one week of fever was taken. The blood samples were sent to the Institute of Vector Control and Zoonoses (**IVCZ**), Hosur, where samples were analyzed for leptospiral antibodies using Microscopic Agglutination Test (MAT). Only one sample was taken from each patient due to non availability of testing centre in Thanjavur and due to delay in obtaining the initial serology report.

During the hospital stay, all the patients were subjected again to liver function tests every week. The renal function tests were repeated every third day unless the patient developed ARF for whom the tests were done daily.

Follow up of all patients regarding treatment and outcome were done during the hospital stay.

RESULTS

Incidence:

- No. of cases admitted with fever and jaundice: 164
- No. of cases found serologically positive for leptospirosis: 40
- ➢ Incidence of leptospirosis among patients admitted with fever and jaundice is **24%**

*(A **positive case** is one with a MAT titre of more than 1:200 among patients presenting with fever and jaundice- as per **CDC** case definition 1997* [1,4] *and National Institute of Communicable Diseases- **NICD** case definition 2001.* [5]*)*

One Patient was positive both for Leptospiral serology and peripheral smear for *Plasmodium falciparum*.

ANALYSIS OF POSITIVE CASES:

Age Distribution:

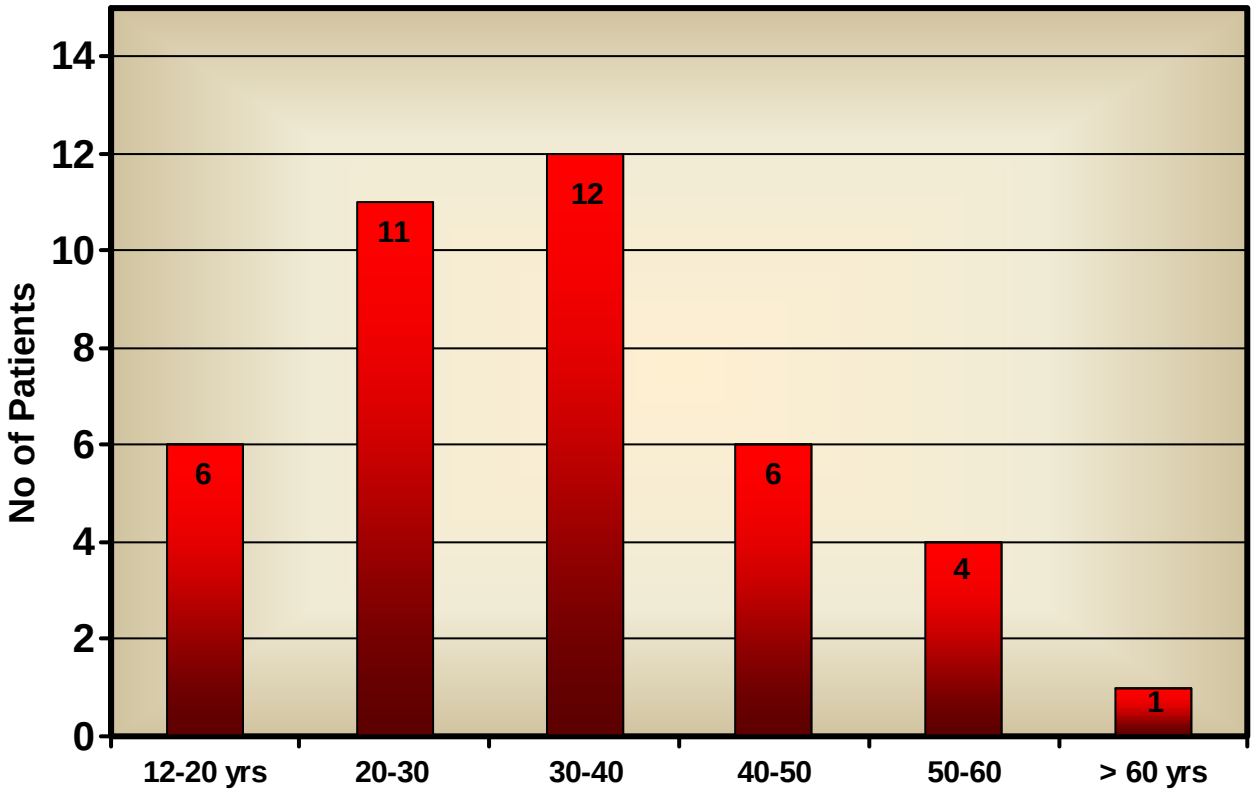
Age	No. of patients	Percentage
12 – 20 years	6	15%
21 – 30 years	11	27.5%
31 – 40 years	12	30%
41 – 50 years	6	15%
51 – 60 years	4	10%
> 60 years	1	2.5%

Sex Distribution:

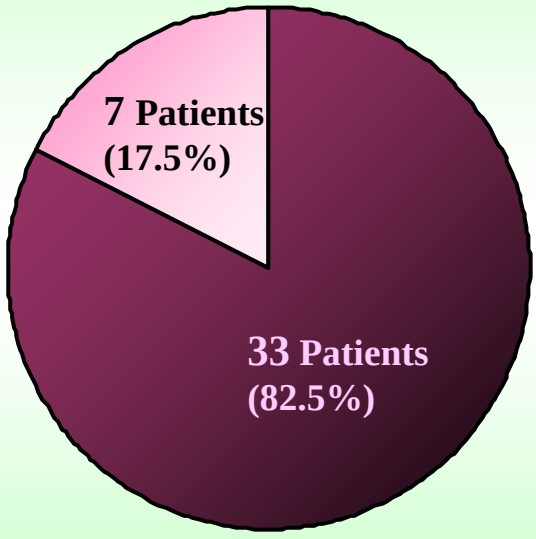
No. of male cases : 33 (82.5%)

No. of female cases : 7 (17.5%)

Age Distribution



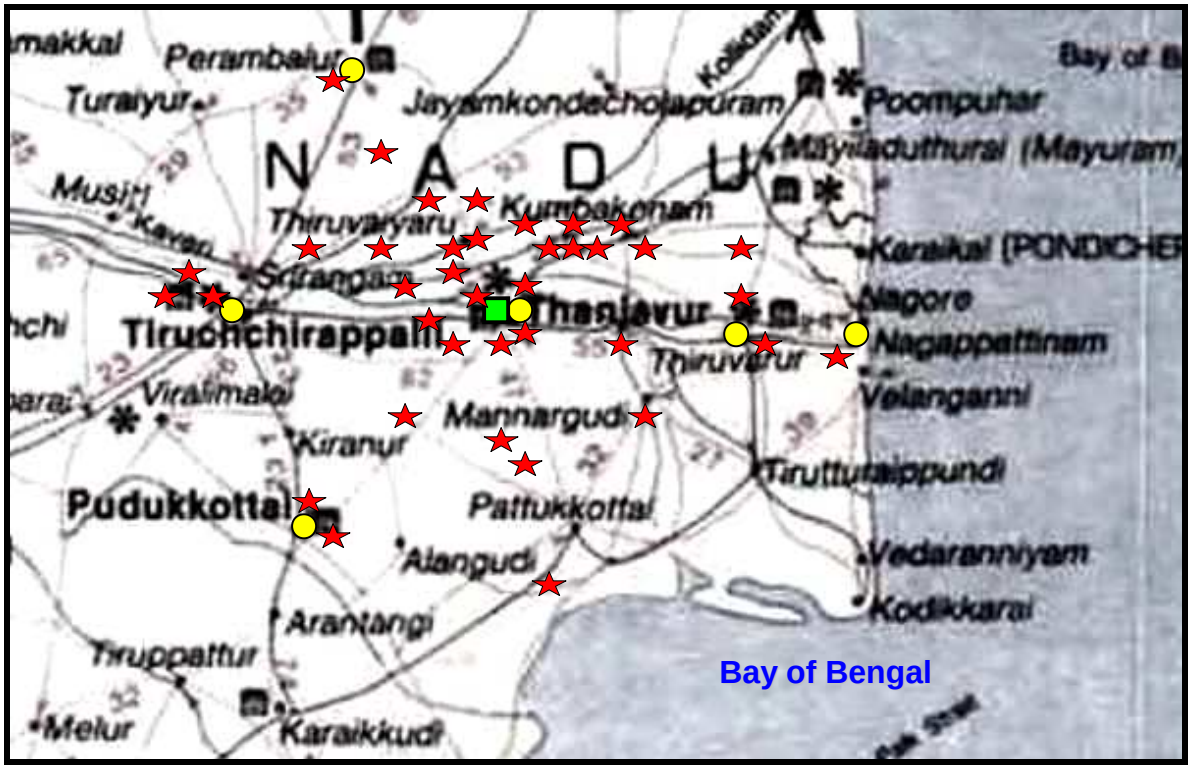
Sex distribution



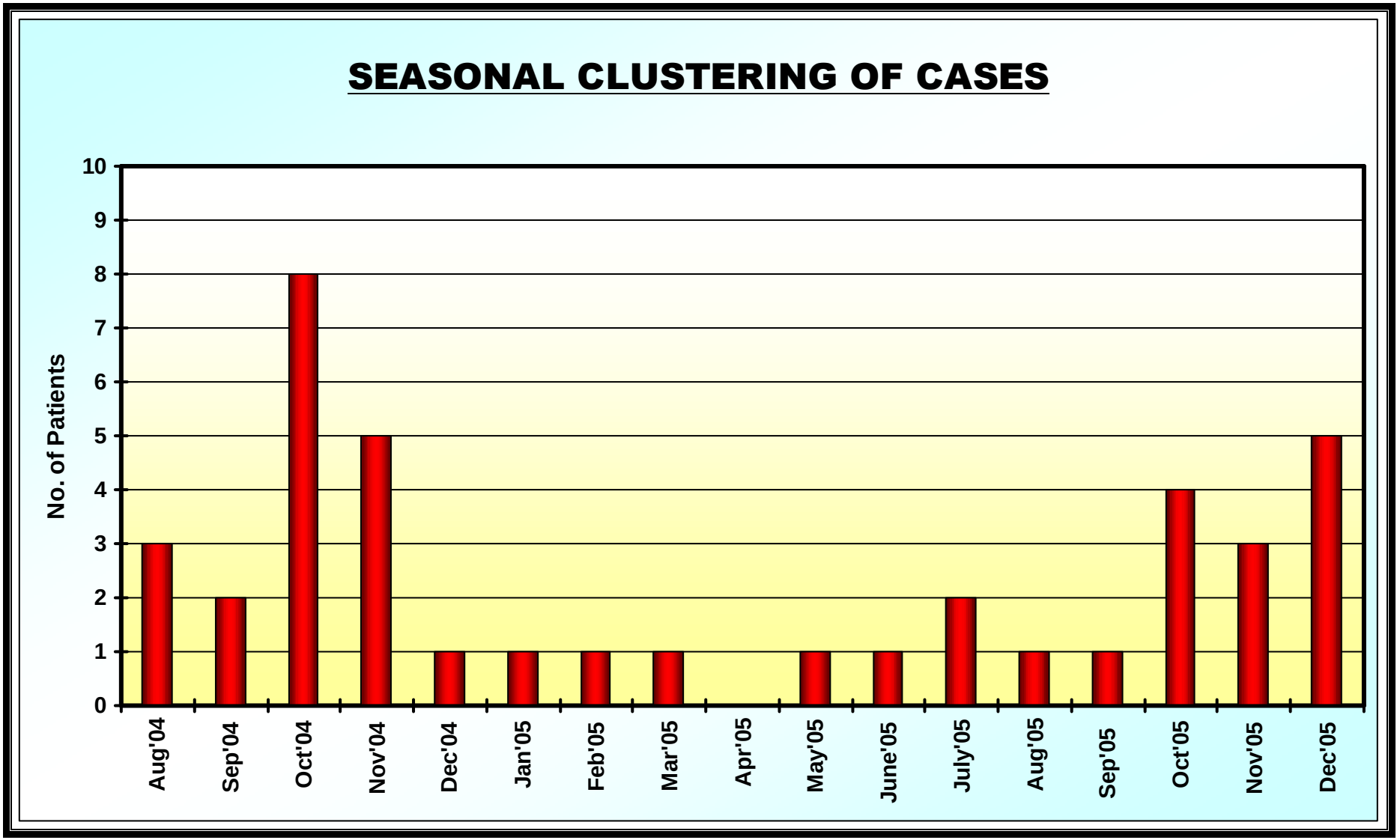
Male Female

DEMOGRAPHIC DISTRIBUTION

Except for two cases which were from Chennai, all the cases were in and around Thanjavur, particularly along the Cauvery and Kollidam rivers



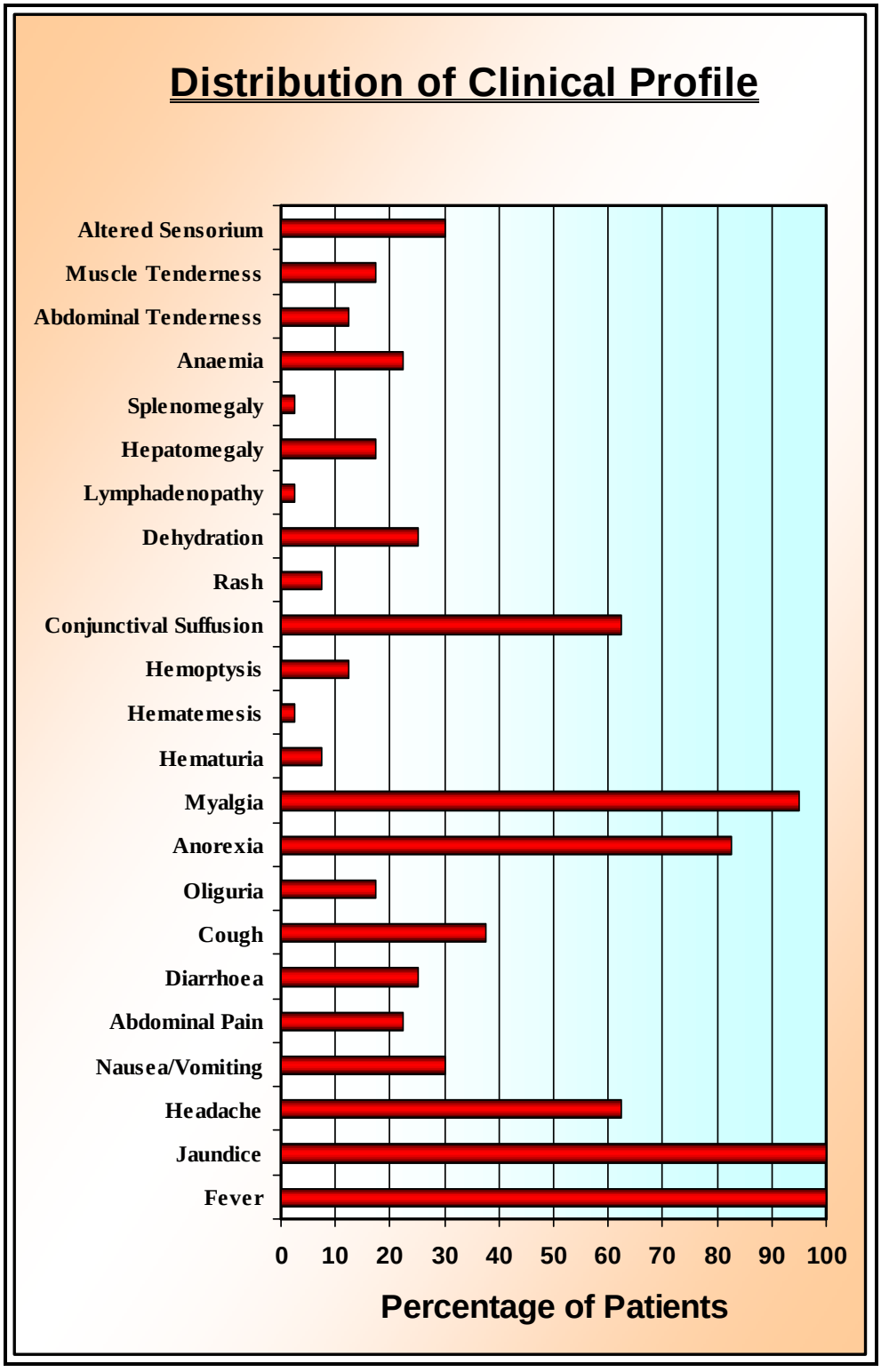
- **Location of Thanjavur Medical College Hospital**
- **District Headquarters**
- ★ **Cases**


SEASONAL CLUSTERING OF CASES
No. of Patients
0
1
2
3
4
5
6
7
8
9
10
Aug'04
Sep'04
Oct'04
Nov'04
Dec'04
Jan'05
Feb'05
Mar'05
Apr'05
May'05
June'05
July'05
Aug'05
Sep'05
Oct'05
Nov'05
Dec'05

DISTRIBUTION OF SIGNS AND SYMPTOMS:

Symptom/Sign	*No. of Patients*	*Percentage*
Fever	40	100%
Jaundice	40	100%
Headache	25	62.5%
Nausea/Vomiting	12	30%
Abdominal Pain	9	22.5%
Diarrhoea	10	25%
Cough	15	37.5%
Oliguria	7	17.5%
Anorexia	31	82.5%
Myalgia	38	95%
Hematuria	3	7.5%
Hematemesis	1	2.5%
Hemoptysis	5	12.5%
Conjunctival Suffusion	25	62.5%
Rash	3	7.5%
Dehydration	10	25%
Lymphadenopathy	1	2.5%
Hepatomegaly	7	17.5%
Splenomegaly	1	2.5%
Anaemia	9	22.5%
Abdominal Tenderness	5	12.5%
Muscle Tenderness	7	17.5%
Meningeal Signs	0	0%
Altered Sensorium	12	30%

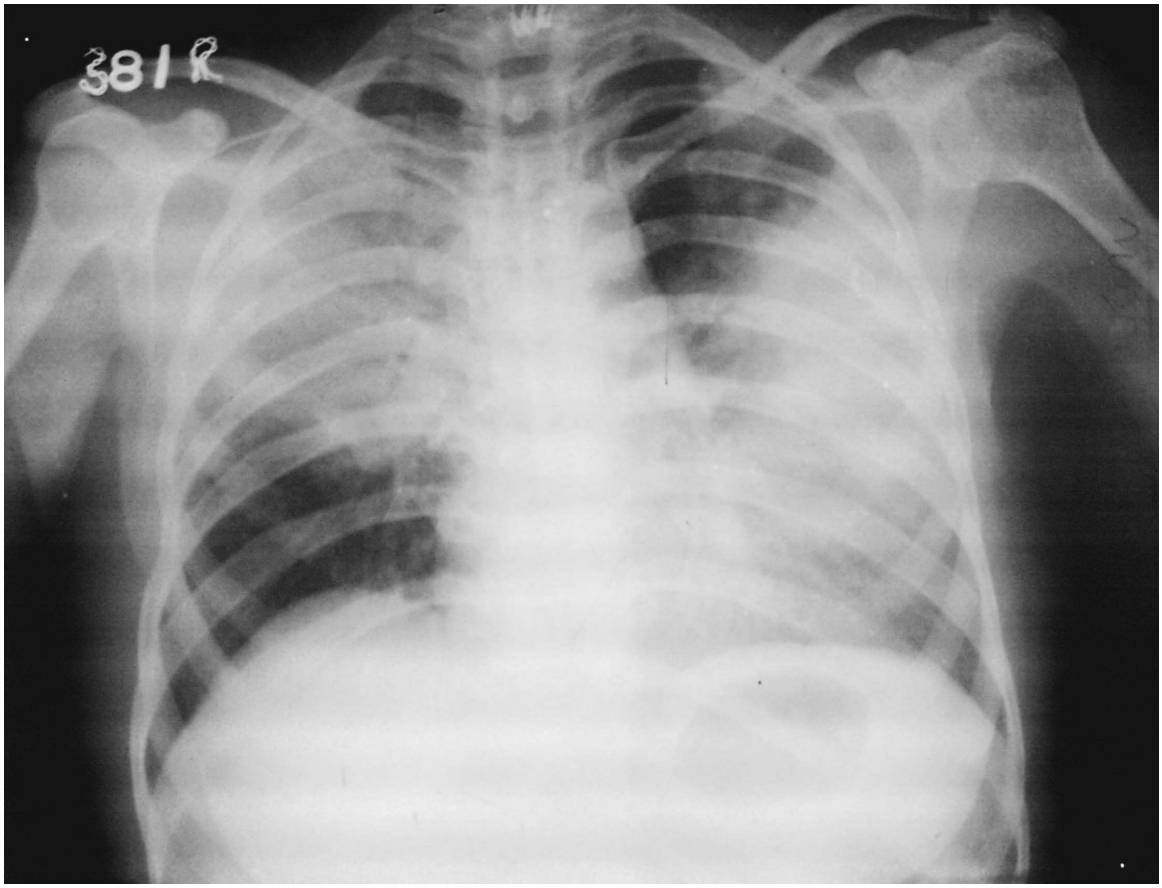
- Average duration of fever on presentation is 6.4 days
- Average duration of jaundice on presentation is 1.6 days


Distribution of Clinical Profile
Altered Sensorium
Muscle Tenderness
Abdominal Tenderness
Anaemia
Splenomegaly
Hepatomegaly
Lymphadenopathy
Dehydration
Rash
Conjunctival Suffusion
Hemoptysis
Hematemesis
Hematuria
Myalgia
Anorexia
Oliguria
Cough
Diarrhoea
Abdominal Pain
Nausea/Vomiting
Headache
Jaundice
Fever
0 10 20 30 40 50 60 70 80 90 100
Percentage of Patients

A male patient with jaundice on initial presentation

A female patient with jaundice on initial presentation

A patient with jaundice and Subconjunctival Hemorrhage



A Leptospirosis patient with Bilateral Pneumonia.

OCCUPATION:

Occupation	No. of Cases	Percentage
Manual Workers	25	62.5%
Non Manual Workers	6	15%
House Wives	2	5%
Students	6	15%
Retired	1	2.5%

ANALYSIS OF LAB INVESTIGATIONS:

- All the seropositive patients were positive for Bile salts and Bile pigments in urine.

Complete Hemogram:

Hemoglobin

- The Mean Hemoglobin values was 9.88 gms%.
- Range – 5.8 to 13.1gms%.
- Six patients had hemoglobin ≤8gms%.

WBC Count:

- The Mean Total Count of WBCs was 8262 cells/µL.
- Range – 3200 to 14,200 cells/µL.

Platelet Count:

- The Mean Platelet Count was 1.63 lakhs/µL.
- Range – 86,000 to 2.32 lakhs/µL.
- Three Patients had Platelet Count <1 lakh/µL.

Erythrocyte Sedimentation Rate:

- Mean ESR at 1 hour was 56 mm.
- Range 16 to 140mm.

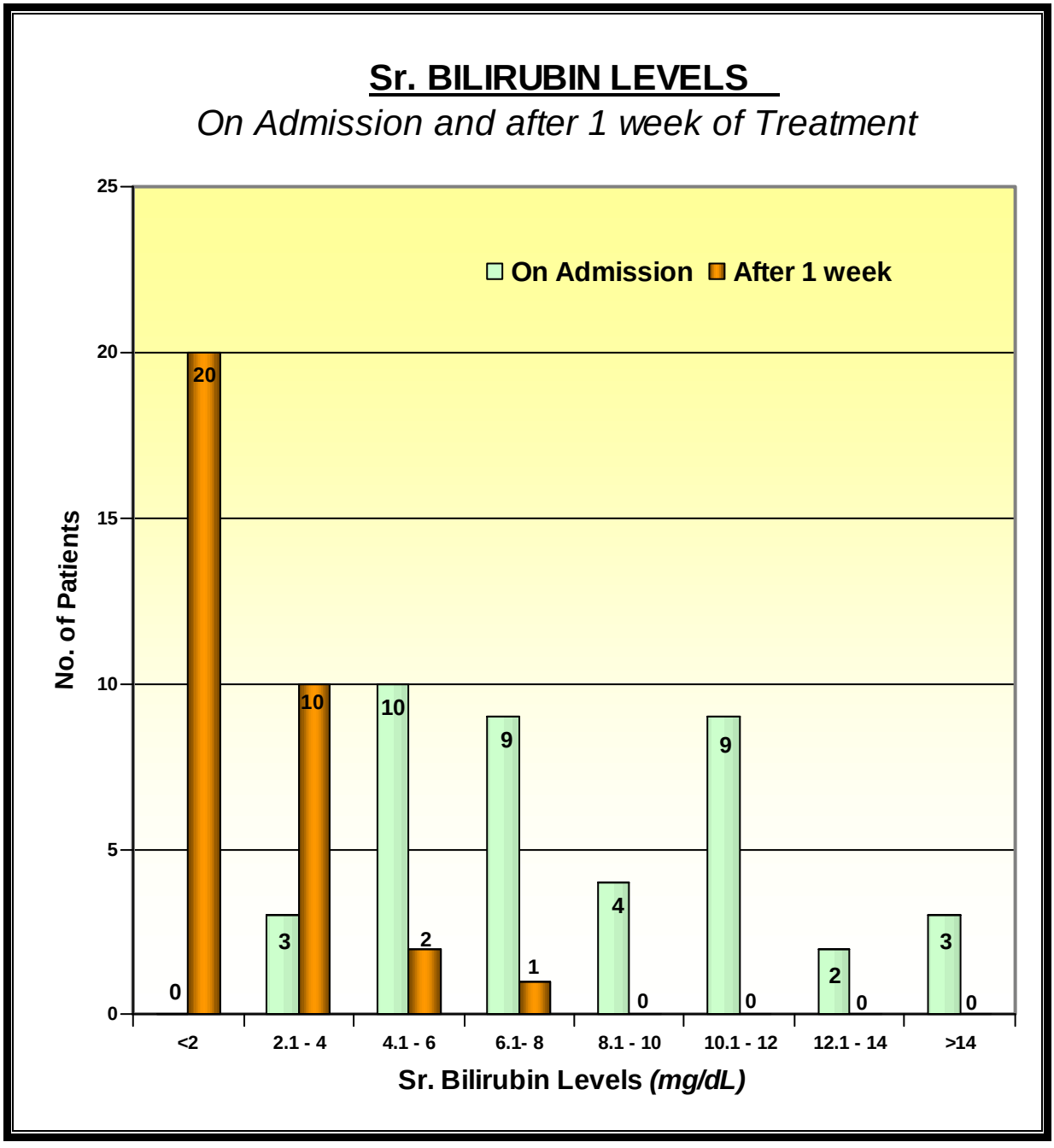
LIVER FUNCTION TESTS: *Bilirubin:*

Total Bilirubin *(mg/dL)*	*On Admission*		*After 1 week of treatment*	
	No. of Patients	Percentage	No. of Patients	Percentage
≤ 2	None	0%	20	50%
2.1 – 4	3	7.5%	10	25%
4.1 – 6	10	25%	2	5%
6.1 – 8	9	22.5%	1	2.5%
8.1 – 10	4	10%	-	-
10.1 – 12	9	22.5%	-	-
12.1 – 14	2	5%	-	-
>14	3	7.5%	-	-

On Admission	**Mean**	**Range**
Total Bilirubin	8.36 mg/dL	3.2 – 28.9 mg/dL
Indirect Bilirubin	3.65 mg/dL	1.4 – 11.7 mg/dL
Direct Bilirubin	4.71 mg/dL	1.1 – 16.1 mg/dL

Other Liver Function Tests:

Tests	**Mean**	**Range**
SGOT *(U/L)*	58.7	22 – 240
SGPT *(U/L)*	57.7	18 – 232
Alkaline Phosphatase *(IU/L)*	116.9	53 – 246
Total Protein *(gms/dL)*	6.4	5.1 – 7.2



- ***Only 33 Patients were alive 1 week after admission.***

RENAL FUNCTION TESTS:

Test	Mean	Range
Blood Urea *(mg/dL)*	51.7	26 to 127
Sr. Creatinine *(mg/dL)*	1.8	0.8 to 7.4

Serum Creatinine levels:

Sr. Creatinine *(mg/dL)*	No. of Patients	Percentage
< 1.5	22	55%
1.5 – 2.0	8	20%
2.1 – 2.5	3	7.5%
>2.5	7	17.5%

Blood Urea levels:

Blood Urea_(mg/dL)	No. of Patients	Percentage
≤ 40	22	55%
41 – 60	7	17.5%
61 – 80	4	10%
81 – 100	4	10%
>100	3	7.5%

- After rehydration, renal parameters returned to normal range except for six patients who needed nephrologist's intervention and dialysis.

Serum Creatine Phospho Kinase (CPK):

- Mean CPK value is 361 U/L. (***Normal < 200 U/L***)
- Range : 90 to 656 U/L

TREATMENT:

- All patients were started on Inj. Benzyl Penicillin 20 Lakhs Units Intravenously – four times daily.
- All patients were started on Penicillin from the day of admission after test dose.
- None of the Patients were allergic to Penicillin.
- Treatment with Penicillin was given for a minimum of 7 days.

END POINTS:

- Whether the Patient is cured and discharged or the Patient succumbed to the illness.
  - No. of Patients discharged after cure : 32
  - No. of Patients Expired : 8.

DURATION OF HOSPITAL STAY:

Among the 32 Patients who were discharged,

- Mean duration of hospital stay was : 9.15 (Range 7 to 21 days)

Among the 8 patients who died,

- Mean duration of hospital stay was : 5.5 days (Range 2 – 18 days)

COMPLICATIONS:

Complication	*No. of Patients*	*No. cured*	*No.Expired*	*Mortality*
Renal Failure *(Persistent after correction of dehydration)*	**7**	**5**	**2**	**40%**
Lung Consolidation	**2**	**2**	**-**	**0%**
Bleeding Disorders *(Hematemesis, Hematuria, Thrombocytopenia, DIC)*	**3**	**1**	**2**	**66%**
Encephalopathy *(Metabolic, Hepatic)*	**9**	**5**	**4**	**44%**

- ***Other complications like Ocular complications, Cardiac complications, etc. were not seen.***

RENAL FAILURE:

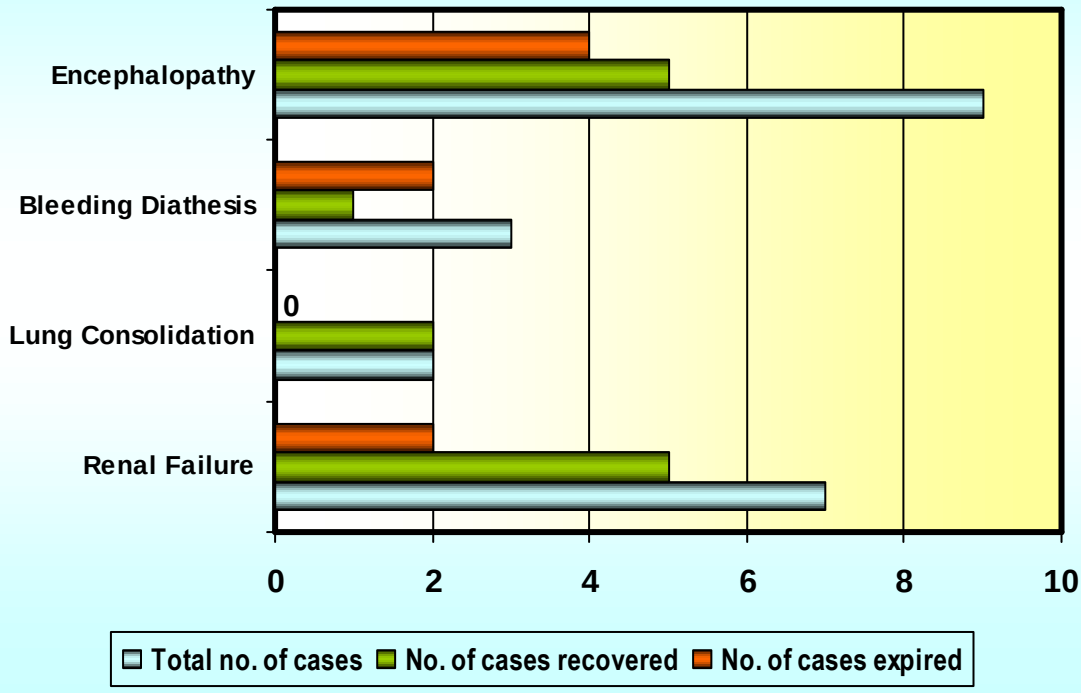
- Oliguria and elevated renal parameters even after rehydration was present in 7 patients
- No. of cases for which Peritoneal Dialysis was Performed : 7 patients
- Normal Renal Function Restored in 5 patients.
- On an average, 2-3 cycles of PD were done per patient.
- No. expired despite treatment: 2 Patients

MORTALITY:

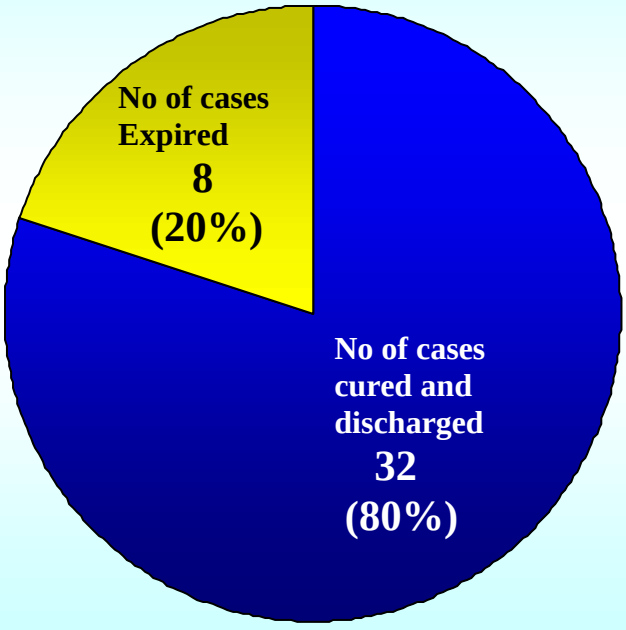
No. of Cases Expired – 8

Mortality rate is 8/40 – 20%

Analysis of Complications



OUTCOME



DISCUSSION

Among the patients admitted with fever and jaundice, the incidence of Leptospirosis was 24%.

Most of the patients were in the working group aged between 20 and 40 years. Further most of them were males (M:F=5:1). This explains that males frequently go out of their homes for work and males also have greater predilection for acquiring the disease (Hormonal factors).[54]

Further the analysis of the occupation reveals that most of them were laborers (62.5%) working in the fields or businessmen with frequent history of travel. Muthusethupathi *et al*[17] gives the incidence among manual labourers as 49%.

The assessment of **Demographic and Seasonal distribution** plays an important role in this study. Thanjavur and its surrounding areas is well known for its rice cultivation (hence called 'Rice Bowl' of Tamilnadu), especially the 'Kuruvai' crops which are cultivated during the months of October to January. It is the period during which Northwest monsoon is active in Tamilnadu.

Though cases occurred in areas scattered in and around Thanjavur, only two cases were from seashore regions like Nagapattinam and Peravurani and four from dry areas like Perambalur, Ariyalur and Pudukottai. Otherwise most of the cases (32 cases) were from areas along Cauvery river and its Delta

distributaries and its main distributary Kollidam – especially from places like Thiruchirapalli, Lalgudi, Thirukattupalli, Thanjavur, Thiruvaiyaru, Thirumanur, Elakurichi, Papanasam, Kumbakonam, Thiruvarur, etc.

Further as far as the Seasonal Distribution of cases were concerned, most of the cases were admitted during the months of October, November and December during which the Northwest monsoon is active in Tamil Nadu though sporadic cases were also seen during other months of the year.

- It is evident from these observations that during the rainy season which usually brings water into the river Cauvery and its branches, incidence of Leptospirosis is greater which attributes that the source of infection is probably the contaminated river water.
- Further it is during this period that ploughing of rice fields, sowing and transplantation of saplings are active in this region which needs prolonged contact with water.
- Hence the disease may not be endemic in this region. The Leptospira infecting the people could be brought by the river water from the upstream areas along the Cauvery River to the delta region. Further analysis is needed to find out the serovars infecting the community.
- Padre *et al*[55] describes increased incidence of Leptospirosis among rice field workers.

ANALYSIS OF CLINICAL FEATURES:

Since this study was performed in patients presenting with fever and jaundice, other clinical symptoms and signs were analyzed on admission.

- **The mean duration of history of Fever was 6.4 days on admission with the range between 4 to 9 days.**
- **The mean duration of history of Jaundice was 1.6 days on admission.**

Until jaundice was noted by the clinician or by themselves, most of the cases were treated as outpatients for fever. Some of them took self treatment with antipyretics.

Among the symptoms other than fever and jaundice, most cases presented with Myalgia (38 cases, 95%), anorexia (31 cases, 82.5%) and headache (25 cases, 62.5%). Rarer symptoms noted in our study were hematuria (7.5%), hematemesis (2.5%), and hemoptysis (12.5%).

Common signs seen during admission were conjunctival suffusion (25 cases, 62.5%), altered sensorium (12 cases, 30%) and dehydration (10 cases, 25%). Rare signs noted were lymphadenopathy (2.5%), splenomegaly (2.5%) and rash (7.5%). The rashes noted were erythematous macules which disappeared within few days of starting treatment.

After treatment, except for cases which expired, all the patients improved clinically. Dehydration was corrected immediately on admission except for cases with oliguria and renal failure. The sensorium also improved in most of the patients on starting treatment.

COMPARISON OF SIGNS AND SYMPTOMS ON ADMISSION

in patients with Leptospirosis

IN LARGE CASE SERIES AND IN THIS STUDY [10, 17, 11, 12,]

(% of patients)

Signs and Symptoms	China[10] 1965 *n = 168*	India[17] 1992 *n = 57*	Brazil[11] 1999 *n = 193*	India[12] 2002 *n = 74*	*In this Study** *n = 40*
Jaundice	0	84	95	34	100
Anorexia	46	85	-	-	82.5
Headache	90	26	75	92	62.5
Conjunctival Suffusion	57	58	28.5	35	62.5
Vomiting	18	58	-	-	30
Myalgia	64	82	94	68	95
Abdominal Pain	26	18	-	-	22.5
Dehydration	-	39	-	-	25
Cough	57	32	-	-	37.5
Hemoptysis	51	9	20	35	12.5
Hematemesis/Melena	-	26	-	-	2.5
Hepatomegaly	28	-	-	-	17.5
Lymphadenopathy	49	-	-	15	2.5
Diarrhoea	20	26	-	-	25
Arthralgia	36	-	-	12	-
Rash	-	-	-	12	7.5
Meningeal signs	-	7	-	-	-
Altered Sensorium	-	42	-	-	30

Though other studies included patients with fever alone, our study included patients presenting with both fever and jaundice.

ANALYSIS OF LABORATORY INVESTIGATIONS:

All the patients in our study who had fever and jaundice on admission were positive for Bile Salts and Bile Pigments in Urine which revealed the presence of Hyperbilirubinemia by bed side.

Complete Hemogram:

Complete Hemogram revealed that most of the patients had normal Hemoglobin levels except for six of them for whom the hemoglobin levels were ≤ 8 gms/dL. Further analysis revealed that among the six patients, all of them presented with hemoptysis, three of them presented with hematuria and one with hematemesis.

The total count of WBCs revealed mild Leucocytosis in four cases (10%) – while in others it was within normal limits. The Differential Count revealed mild Neutrophilia in three cases (7.5%).

Most of them had normal Platelet Count. Only in three patients (7.5%), the Platelet Count was less than 1 lakh/μL. All the three patients presented with bleeding manifestations.

There was a mild rise in Erythrocyte Sedimentation Rate in most of the patients. Five (12.5%) of the patients had ESR above 100mm in 1 hour.

It is evident from these observations that non-specific changes in Hemogram can occur in Leptospirosis. Though they are not useful in the diagnosis of disease, they are helpful in suspicion of the disease.

Liver function tests:

The Analysis of LFT reveals a high level of Bilirubin on admission ranging from 3.2 to 28.9 mg/dL. There was a Conjugated Hyperbilirubinemia in most of the cases. Almost all of them who responded to treatment had marked reduction in their Bilirubin levels after 1 week.

There was mild increase in Serum Transaminase levels while there was a moderate increase in Serum Alkaline Phosphatase levels. The Total Protein level was within normal limits. Further follow-up of the patients during the hospital stay revealed that the enzyme levels returned to normal on treatment.

Renal Function Tests:

While only seven patients presented with oliguria, laboratory investigations revealed mild to severe elevation of Blood Urea and Serum Creatinine levels in 45% of patients. Among them, 10 patients (25%) had serum creatinine levels of more than 2mg/dL and 11 (27.5%) patients had Blood Urea level of more than 60mg/dL including three of them with levels more than 100mg/dL.

Further it was noted that after Rehydration with oral or intravenous fluids, most of the patients with mild raise in Renal Parameters returned to normal levels except in seven patients (17.5%) for whom Nephrologist's intervention was needed.

There was a mild to moderate elevation of ***Serum Creatine Phospho Kinase*** levels during admission for 32 (80%) patients, which reveals the acute nature of the disease. The maximum value was 656 U/L (normal – less than 200 U/L).

ABNORMAL BIOCHEMICAL VALUES:

Comparison with a study conducted in Chennai

Parameter	Abnormal values	**Muthusethupathi *et.al*** [17] *n = 57*		**In our study** *n = 40*	
		No. of cases	**Percentage**	**No. of cases**	**Percentage**
Blood Urea	>40 mg/dL	41	72	18	45
Sr.Creatinine	>1.5 mg/dL	41	72	18	45
Bilirubin	>2.0 mg/dL	48	82	40	100
SGOT	>40 U/L	25	44	21	52.5
SGPT	>40 U/L	27	47	22	55
Platelets	< 1 lakh/µL	13	23	3	7.5

Co-infection with Pl. falciparum Malaria was seen in one case in this study. This coincides with various studies on Leptospirosis with concomitant illnesses, viz. Malaria[46], Hepatitis, Dengue[34], Melioidosis, Scrub typhus, etc. Wongsrichanalai *et al* [46] reported two cases of dual infection with malarial parasites and leptospira. One patient was infected with Plasmodium falciparum and the other with P. vivax.

The outcome of the treatment revealed that all the patients were sensitive to Benzyl Penicillin injection given intravenously. Most of the patients also received a presumptive treatment with Chloroquine for Malaria. Only one patient was proved to have multiple infections with Leptospira and Plasmodium falciparum malaria.

The common complications were mostly renal failure (7 cases) with some presenting with altered sensorium (12 cases). Moreover outcome was good in patients with renal failure with death occurring in 2 out of seven cases (28%) whereas among the three patients who presented with bleeding disorders, the mortality was 66%. Among the 2 cases which presented with features of consolidation, with chest X-Ray showing Homogenous Opacities, the outcome was excellent with both cases getting cured and discharged. Renal failure patients received Peritoneal Dialysis with good results (5 out of 7 cured). Muthusethupathi *et al*[14] too reveals that peritoneal dialysis is satisfactory. Other complications including Cardiac complications like arrhythmias, myocarditis, Ocular complications like Uveitis, Cerebrovascular Accident, Acalculous Cholecystitis, Arthritis, GBS, etc. – which are described in textbooks [1,2,3,7] were not noted in this study.

This study also reveals a high mortality rate for complicated Icteric leptospirosis of 20% (8cases). Levett PN [1] gives the mortality rate as 5-15%.

CONCLUSION

1) **The incidence of Leptospirosis among adults admitted with fever and jaundice was 24%.**
2) **Most of the cases (80%) were from the places along the Cauvery and Kollidam rivers and their Delta distributaries.**
3) **Most of the cases occurred during the monsoon months of October, November and December.**
4) **The infection may not be endemic to this region but could be brought from upstream areas of Cauvery River during rainy season.**
5) **Most of the cases were manual workers (62.5%).**
6) **Apart from fever and jaundice, the most common symptoms/signs present during admission in patients with Leptospirosis were Myalgia, Anorexia, Headache and Conjunctival Suffusion.**
7) **Mixed infection of Leptospirosis with *Plasmodium falciparum* was seen in one case.**

8) **All the cases had hyperbilirubinemia mostly of conjugated type.**

9) **Most of them had prerenal azotemia which was corrected by rehydration.**

10) **Mild to moderate elevation of CPK levels was seen in 80% cases.**

11) **All alive patients responded well to intravenous Penicillin therapy.**

12) **Seven cases (17.5%) went for Intrinsic Acute Renal Failure with five of them recovering with Peritoneal Dialysis.**

13) **The overall mortality of the Icteric Leptospirosis cases was 20%.**

BIBLIOGRAPHY

1) **Levett PN. Leptospirosis.** *Clinical Microbiology Reviews*, April 2001, p. 296-326, Vol. 14, No. 2
2) **Levett PN. Leptospirosis.** *In* : Mandell GL, Bennett JE, Dolin R, editors. ***Principles and Practice of Infectious Diseases*** . 6th edn. Philadelphia: Elsevier Churchill Livingstone; 2005. p. 2789-95.
3) **Speelman Peter**. Leptospirosis. *In*: Braunwald, Fauci, Kasper, Hauser, editors. ***Harrison's Principles of Internal Medicine***. 16th edn. New York: McGraw Hill Publications; 2005. p.988-991
4) **Centers for Disease Control and Prevention**. 1997. Case definitions for infectious conditions under public health surveillance. *Morb. Mortal. Wkly*. Rep. 46(RR-10):49.
   Website:http://www.cdc.gov/ncidod/dbmd/diseaseinfo/leptospirosis.html.
5) **National institute of communicable diseases (NICD).** Case definitions of epidemic prone diseases. DGHS, Delhi; 2001.
   Website:http://www.nicd.org/AllDisease.asp#11.
6) **Richard A. Jacobs**. Leptospirosis. *Chapter in*: Lawrence M. Tierney, Stephen J. McPhee, Maxine A. Papadakis, editors. ***CURRENT Medical Diagnosis and Treatment 2006***, 45th Edition, Lange/McGraw-Hill 2006. p:1455-1456.
7) **Dilip R. Karnad**. Leptospirosis. *Chapter in* : Siddharth N Shah (Edited), ***API Text book of Medicine***, 7th edition, 2003. Association of Physicians of India, Mumbai. Page 73-74.
8) **Park K**. Park's Text Book of preventive and social medicine. 18th edition 2005. Banarsidas Bhanot, Jabalpur. Page 232-233.

9) **Dutta TK, Christopher M**. Leptospirosis - an overview. *J Assoc Physic Ind* 2005;53:545-51.

10) **Wang, C., L. John, T. Chang, W. Cheng, M. Luo, and A. Hung**. 1965. Studies on anicteric leptospirosis. I. Clinical manifestations and antibiotic therapy. *Chin. Med. J.* 84:283-291.

11) **Ko, A. I., M. Galvao Reis, C. M. Ribeiro Dourado, W. D. Johnson, L. W. Riley, and the Salvador Leptospirosis Study Group**. 1999. Urban epidemic of severe leptospirosis in Brazil. *Lancet* 354:820-825.

12) **Bharadwaj R, Bal AM, Joshi SA, Kagal A, Pol SS, Garad G**, *et al* . An urban outbreak of leptospirosis in Mumbai, India. *Jpn J Infect Dis* 2002;55:194-6.

13) **Weyant, R. S., S. L. Bragg, and A. F. Kaufman**. 1999. *Leptospira* and *Leptonema*, p. 739-745. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.

14) **Muthusethupathi, MA., and S. Shivakumar.** 1987. Acute renal failure due to leptospirosis. *J. Assoc. Phys. India* 35:631-633.

15) **Muthusethupathi MA, Shivakurnar S, Jayakumar M, Rajendran S**. Acute Renal failure in Madras city. Changing profile. *Ind J Nephrol* (New Series) 1993; 3:66-70.

16) **Muthusethupathi MA, Shivakumar S, Suguna Rajendran, Vijayakumar R, Jayakumar M**. Leptospiral renal failure in Madras city. *Ind J Nephrol* 1991; 1:15-17.

17) **Shivakumar S, Muthusethupathi MA, Everard COR**. Leptospirosis in Madras city - A collaborative study (abstract). *V Asian Pacific Congress of Nephrology* 1992; 44

18) **Shivakumar S, Shareek PS**. Diagnosis of leptospirosis utilizing modified Faine's criteria. *J Assoc Physicians India* 2004;52:678-9

19) **Clerke AM, Leuva AC, Joshi C, Trivedi SV**. Clinical profile of leptospirosis in South gujarat. *J Postgrad Med* 2002;48:117-8

20) **Ahmad SN, Shah S, H Ahmad FM**. Laboratory diagnosis of leptospirosis. *J Postgrad Med* Year 2005, Volume 51, Issue 3, page 195-200.

21) **Shah I, Warke S, Deshmukh CT, Kamat JR.** Leptospirosis - an under-diagnosed clinical condition. *J Postgrad Med* 1999;45:93-4

22) **Visith S, Kearkiat P.** Nephropathy in leptospirosis. *J Postgrad Med* 2005;51:184-188

23) **Salkade HP, Divate S, Deshpande JR, Kawishwar V, Chaturvedi R, Kandalkar BM, Vaideeswar P**. A study of autopsy findings in 62 cases of leptospirosis in a metropolitan city in India. *J Postgrad Med* 2005;51:169-173

24) **Bal AM**. Unusual clinical manifestations of leptospirosis. *J Postgrad Med* 2005;51:179-183

25) **Ratnam S, Everard COR, Alex JC, Suresh B, Thangaraju P**. Prevalence of leptospiral agglutinins among conservancy workers in Madras city, India. *J Trop Mad and Hygiene* 1993; 96:41-45.

26) **Ratnam S, Sundararaj T, Subramaniam S**. Role of bandicoots in human leptospirosis in Madras City - An epidemiological approach. *Ind Pub Health 1986*; 30:1678

27) **Watt G, Tuazom MA, Santiago LE**. Placebo controlled trial of intravenous penicillin for severe and late teptospirosis *Lancet* 1988; 1:433-435

28) **Cumberland PC, Everard CO, Levett PN**. Assessment of the efficacy of the IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. *Am J Trop Med Hyg* 1999;61:731-4

29) **Brandyo AP, Camargo ED, da Silva ED, Silva MV, Abrγo RV**. Microscopic agglutination test for rapid diagnosis of human leptospirosis. *J Clin Microbiol* 1998;36:3138-42.

30) **Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, *et al*** . Leptospirosis:A zoonotic disease of global importance. *Lancet Infect Dis* 2003;3:757-71. [PMID: 14652202]

31) **Vijayachari V, Sugunan AP, Murhekar MV, Sharma S, Sehgal SC**. Leptospirosis among schoolchildren of the Andaman & Nicobar Islands, India: Low levels of morbidity and mortality among pre-exposed children during an epidemic. *Epidemiol Infect*. 2004;132:1115-20.

32) **Sethi S, Sood A, Pooja, Sharma S, Sengupta C, Sharma M**. Leptospirosis in northern India:a clinical and serological study. *Southeast Asian J Trop Med Public Health* 2003;34:822-5.

33) **Bal AM, Kakrani AL, Bharadwaj RS, Kagal AS, Joshi SA, Arjunwadkar VP**. Evaluation of clinical criteria for the diagnosis of leptospirosis. *J Assoc Physic Ind* 2002;50:394-6.

34) **Karande S, Gandhi D, Kulkarni M, Bharadwaj R, Pol S, Thakare J, *et al*** . Concurrent Outbreak of Leptospirosis and Dengue in Mumbai, India, 2002. *J Trop Pediatr* 2005;51:174-81

35) **Perani V, Farina C, Maggi L, Michetti G, Moioli F, Pizzocaro P, *et al***. Pneumonia due to Leptospira spp.: results of an epidemiological and clinical study. *Int J Tuberc Lung Dis* 1998;2:766-70

36) **Sehgal SC, Murhekar MV, Sugunan AP**. Outbreak of leptospirosis with pulmonary involvement in north Andaman. *Indian J Med Res* 1995;102:9-12.

37) **Karande S, Satam N, Kulkarni M, Bharadwaj R, Pol S**. Leptospiral pneumonia. *Indian J Pediatr* 2005;72:86

38) **Rathinam SR, Rathnam S, Selvaraj S, Dean D, Nozik RA, Namperumalsamy P**. Uveitis associated with an epidemic outbreak of leptospirosis. *Am J Ophthalmol* 1997;124:71-9.

39) **Sitprija V, Pipatanagul V, Mertowidjojo K, Boonpucknavig V, Boonpucknavig S**. Pathogenesis of renal disease in leptospirosis. *Kidney Int* 1980;17:827-36.

40) **Sitprija V, Losuwanrak K, Kanjanabuch T**. Leptospiral nephropathy. *Semin Nephrol* 2003;23:42-8.

41) **Singh SS, Vijajachari P, Sinha A**. Clinico-epidemiological study of hospitalised cases of severe leptospirosis. *IndianJMed Res* 1999;109:94-9

42) **Rajiv C, Manjuran RJ, Sudhayakumar N, Haneef M**. Cardiovascular involvement in leptospirosis. *Indian Heart J* 1996;48:691-4.

43) **Krishnamurthi MV, Madanagopalan N, Hussain AT**. Leptospirosis in Madras. *J Assoc Phys India* 1965;13: 737-740.

44) **Sehgal SC**. Emergence of leptospirosis as a public health problem. *Proceedings of the round table conference on leptospirosis*; Pune 1998; 3: 7-17

45) **Murhekar MV, Sugunan AP, Vidyachari P, Sharma S, Sehgal SC**. Risk factors in the transmission of leptospiral infection. *Indian Journal of Medical Research 1998*; 107 : 218-23.

46) **Wongsrichanalai C, Murray CK, Gray M, Miller RS, McDaniel P, Liao WJ, *et al*** . Co-infection with malaria and leptospirosis. *Am J Trop Med Hyg* 2003;68:583-5

47) **Katz AR et al**: Assessment of the clinical presentation and treatment of 353 cases of laboratory-confirmed leptospirosis in Hawaii, 1974-1998. *Clin Infect Dis* 2001;33:1834. [PMID: 11692294]

48) **Vintez JM**: Leptospirosis. *Curr Opin Infect Dis* 2001;14:157. [PMID: 11964872]

49) **Scott G, Coleman TJ**: Leptospirosis. *In:* Gordon C Cook, Alimuddin Zumla- Editors, ***Manson's Tropical Diseases***, 21st Edition,Saunders; 2003. Page 1165-1171.

50) **Anantha Narayanan and Paniker's** ***Textbook of Microbiology***, 7th Edition, Orient Longman 2005. Page 389-393.

51) **Sehgal SC, Sugunan AP, Murhekar MV, Sharma S & Vijayachari P.** Randomized controlled trial of Doxycycline prophylaxis against leptospirosis in an endemic area. *Int J Antimicrob Agents* 2000;13: 249-255.

52) **Ramadass P, Samuel B & Nachimuthu K.** A rapid latex Agglutination test for detection of leptosppiral antibodies. *Vet Microbiol* 1999;70: 137-140.

53) **Sheila Sherlock** and **James Dooley.** Leptospirosis. *In:* Diseases of the liver and biliary system, 11th Edition, Blackwell Publishers, 2002: pages 504-507.

54) **Cachay ER, Vinetz JM**. A global research agenda for leptospirosis. *J Postgrad Med* 2005;51:174-178

55) **Padre,L.P., G.Watt, M.L.Tuazon, M.R.Gray,** and **L.W.Laughlin.** 1988. A serologic survey of rice-field leptospirosis in central Luzon, Philippines. *Southeast Asian J. Trop. Med. Public Health* **19:**197-199

PROFORMA

NAME:	**Age**:	**IP No:**
Date of Admission:	**Sex: M□**	**F□**
Ward/ Unit:		
Address:		

Occupation: □ Manual Worker

□ Non Manual Worker

□ House wife

□ Student

□ Retired

SYMPTOMS and SIGNS:

□ Jaundice: ____days	□ Fever: ____days	□ Headache
□ Nausea / Vomiting	□ Abdominal Pain	□ Diarrhoea
□ Cough	□ Hemoptysis	□ Anorexia
□ Conjunctival Suffusion	□ Myalgia	□ Chills
□ Oliguria /Anuria	□ Haematuria	

□ Rash	□ Dehydration	□ Lymphadenopathy
□ Hepatomegaly	□ Anaemia	□ Splenomegaly
□Abdominal Tenderness	□ Muscle tenderness	
□ Meningeal signs	□ Altered sensorium	
□ Others:		

INVESTIGATIONS:

Serology for Leptospirosis:

Urine: Albumin:

Sugar :

Deposits:

Bile Salts:

Bile Pigments:

Hemogram:

Hb : g %

TC : cells/µL

DC : P: , L: , E: %

Platelets : cells/µL

RBC : cells/µL

ESR : ½ hr: mm

: 1 hr: mm

Blood Biochemistry:

Sr. CPK : U/L

Parameters		Day 1	Rpt	Rpt
Blood Sugar	***mg/dL***			
Blood Urea	***mg/dL***			
Sodium	***mEq/L***			
Potassium	***mEq/L***			

Liver Function Tests:

Date:		Day 1	After 1 week
Total Bilirubin	***mg/dl***		
Direct Bilirubin	***mg/dl***		
Indirect Bilirubin	***mg/d***l		
SGOT	***U/L***		
SGPT	***U/L***		
Sr.Alkaline Phosphatase:	***IU/L***		
Total Protein	***g/d***L		

Ultrasound Abdomen:

Chest X Ray:

ECG :

Other Investigations:

TREATMENT GIVEN:

CLINICAL OUTCOME:

☐ Cured and Discharged

☐ Expired

Duration of stay in hospital: ____days.

76

MASTER CHART

S. No.	NAME	AGE	SEX	PLACE	IP No	Occupation*	DOA	Duration of fever (Days)	Duration of jaundice(Days)	Headache	Nausea/Vomiting	Abdominal Pain	Diarrhoea	Cough	Oliguria	Anorexia	Myalgia	Hematuria	Hematemesis	Hemoptysis	Conjunctival Suffusion	Rash	Dehydration	Lymphadenopathy	Hepatomegaly	Splenomegaly	Anaemia	Abdominal Tenderness	Muscle Tenderness	Altered Sensorium	Serology for Leptospirosis
1	ARIVAZHAGAN	38	M	Lalgudi	801557	MW	6.8.04	5	2	√				√		√	√				√		√								Positive
2	PRAKASH	13	M	Papanasam	803002	S	12.8.04	4	1				√				√														Positive
3	SAKTHIVEL	31	M	Trichy	804241	MW	26.8.04	9	2	√	√	√			√	√	√	√	√	√	√						√	√		√	Positive
4	BASKAR	25	M	Papanasam	811053	MW	17.9.04	5	1					√		√	√														Positive
5	JAWAHAR	22	M	Nagapattinam	812330	MW	28.9.04	7	3	√			√	√		√	√				√										Positive
6	NAGARAJ	47	M	Papanasam	816858	NMW	3.10.04	8	3	√	√	√			√	√	√				√		√		√					√	Positive
7	CHITRA	24	F	Peravurani	817253	HW	11.10.04	5	2	√	√			√			√				√				√	√	√				Positive
8	NATESAN	47	M	Orathanadu	817233	MW	6.10.04	6	1						√	√	√														Positive
9	SASIKUMAR	25	M	Thiruvaiyaru	817693	MW	10.10.04	6	1	√				√		√															Positive
10	ASAITHAMBI	44	M	Nannilam,Thiruvarur	817320	NMW	8.10.04	5	2				√				√				√										Positive
11	CHINNADURAI	35	M	Kumbakonam	817830	MW	11.10.04	7	1	√	√	√				√	√			√	√	√	√		√		√			√	Positive
12	CHELLADURAI	45	M	Vallam	819103	MW	21.10.04	5	1								√														Positive
13	DHAYANIDHI	21	M	Orathanadu	820012	MW	29.10.04	8	3	√						√	√														Positive
14	RAJESH	31	M	Pulivalam,Thiruvarur	820431	MW	4.11.04	7	2	√						√	√				√										Positive
15	SIVARAJ	15	M	Papanasam	820729	S	9.11.04	6	1						√	√	√				√										Positive
16	NEELA	25	F	Pudukottai	821346	MW	15.11.04	7	2	√				√		√	√														Positive
17	RAJENDRAN	37	M	Thirumanur	821941	MW	19.11.04	8	2	√	√	√	√			√	√			√	√		√		√		√			√	Positive
18	SHANMUGAVEL	28	M	Thiruvarur	822293	MW	22.11.04	9	3	√	√	√	√				√						√		√		√			√	Positive
19	SIVAKUMAR	40	M	Trichy	823645	NMW	5.12.04	7	1	√	√		√	√		√	√				√		√	√			√	√		√	Positive
20	MURUGAIYAN	50	M	Thanjavur	829373	NMW	22.1.05	7	1							√	√				√										Positive
21	HARINI	28	F	Thanjavur	832491	HW	16.2.05	6	1	√							√														Positive
22	HARIHARAN	31	M	Thanjavur	838674	MW	11.3.05	5	2					√		√	√				√										Positive
23	MALATHI	17	F	Kumbakonam	840363	S	19.5.05	8	3	√	√	√	√				√	√		√	√	√	√		√		√	√		√	Positive
24	SANTHANAKRISHNAN	60	M	Trichy	840832	NMW	11.6.05	6	1							√	√				√										Positive
25	RAJENDRAN	40	M	Thirukattupalli	849906	MW	7.7.05	4	1	√	√	√	√		√	√	√	√					√							√	Positive
26	KUMAR	35	M	Thanjavur	853482	MW	6.8.05	5	1					√		√					√										Positive
27	VIVEKANANDAN #	38	M	Chennai	855517	NMW	21.9.05	9	3	√						√	√				√										Positive
28	KALIYAPERUMAL	55	M	Mannargudi	861659	MW	5.10.05	6	1						√	√	√				√										Positive
29	JOHN	47	M	Perambalur	863138	MW	19.10.05	7	1	√							√														Positive
30	AROKYARAJ	23	M	Elakurichi	863520	MW	23.10.05	8	2	√	√	√	√				√			√	√	√	√				√	√		√	Positive
31	CHANDRAKALA	25	F	Kantharvakottai	863135	MW	19.10.05	6	1							√	√														Positive
32	VELU	70	M	Thiruvaiyaru	864738	R	5.11.05	7	2	√				√		√	√				√										Positive
33	MATHANKUMAR	19	M	Thiruvaiyaru	865341	S	18.11.05	6	1	√							√														Positive
34	RAJENDRAN	40	M	Thanjavur	867920	MW	28.11.05	5	1					√		√	√				√										Positive
35	MANI	60	M	Ariyalur	869365	MW	18.12.05	6	1		√					√	√														Positive
36	JAMUNA	12	F	Thanjavur	870012	S	19.12.05	5	1							√	√				√										Positive
37	RAVICHANDRAN	35	M	Pudukottai	863280	MW	12.12.05	5	1	√				√		√	√				√		√							√	Positive
38	REXCY	18	F	Chennai	869545	S	23.12.05	7	2	√				√		√	√														Positive
39	ALEXANDER	30	M	Thanjavur	870116	MW	24.12.05	8	2	√	√	√	√			√	√				√		√					√		√	Positive
40	KALYANASUNDARAM	52	M	Needamangalam	851679	MW	21.7.05	6	2	√				√	√	√	√				√				√		√			√	Positive

***MW**:Manual Worker, **NMW**:Non Manual Worker, **HW**:House Wife, **S**:Student, **R**:Retired # also positive for *Plasmodium falciparum* in peripheral smear

S. No.	NAME	HEMOGRAM				LIVE	
		Hb (g%)	Total Count (cells/µL)	Platelet Count (Lakhs)	ESR at 1hr (mm)	Total Bilirubin (mg/dL)	Direct Bilirubin
1	ARIVAZHAGAN	11.2	5400	1.2	122	8.6	4
2	PRAKASH	10.4	7100	0.96	16	14.2	8.4
3	SAKTHIVEL	7.8	13000	1.82	28	4.2	2
4	BASKAR	13.1	7800	2.1	44	6.8	4.2
5	JAWAHAR	11.2	6400	1.6	62	11.2	6.2
6	NAGARAJ	10.4	9600	2.2	52	11.4	6.1
7	CHITRA	8	12600	2.12	24	10.2	5.4
8	NATESAN	9.6	9600	1.4	28	4.8	3
9	SASIKUMAR	9.4	3200	1.6	122	9.2	4.1
10	ASAITHAMBI	11.2	7200	2.32	32	4.2	2.1
11	CHINNADURAI	7.8	13300	0.86	64	6.8	5
12	CHELLADURAI	12.6	6400	1.62	56	11.2	7.1
13	DHAYANIDHI	12.8	9100	1.7	32	10.4	5.8
14	RAJESH	10.2	8200	1.42	32	6.8	3.8
15	SIVARAJ	9.8	6100	2.4	84	5.4	3.4
16	NEELA	9	5400	1.45	72	3.8	1.8
17	RAJENDRAN	8.1	11600	1.62	90	8.4	5.1
18	SHANMUGAVEL	7.2	9600	1.71	22	10.2	5.1
19	SIVAKUMAR	7.8	10400	2	70	3.2	1.1
20	MURUGAIYAN	9	6100	1.32	126	4.2	2.8
21	HARINI	9.8	5600	1.64	32	6.4	3
22	HARIHARAN	11.2	11700	1.72	18	4.2	2.1
23	MALATHI	6.2	14200	1.1	24	3.8	2.1
24	SANTHANAKRISHNAN	12.6	3100	1.2	32	6.6	3.6
25	RAJENDRAN	11.2	5600	0.98	64	4.2	2.2
26	KUMAR	12.6	7200	1.6	96	12.1	8
27	VIVEKANANDAN	8.8	5400	1.32	84	10.4	6
28	KALIYAPERUMAL	10.6	6200	1.4	22	28.9	16.1
29	JOHN	11.2	9100	1.62	18	17.2	11.1
30	AROKYARAJ	5.8	11700	1.25	140	5.6	2.1
31	CHANDRAKALA	10.8	7100	1.62	64	9.6	5.2
32	VELU	10	4400	1.74	110	7.2	4.1
33	MATHANKUMAR	8.8	3600	2.1	32	11.2	5.1
34	RAJENDRAN	11.2	9100	2.2	16	13.6	7.1
35	MANI	9.6	12200	1.82	30	6.8	3.2
36	JAMUNA	10.4	8900	1.6	62	5.4	4
37	RAVICHANDRAN	9.9	9600	1.5	62	6.4	3.2
38	REXCY	9.4	11000	2.1	18	5.2	3.1
39	ALEXANDER	8.1	8900	1.8	51	7.4	4.2

40	KALYANASUNDARAM	10.2	7800	1.6	96	11.6	6.4

MASTER CHART

ER FUNCTION TESTS								
Indirect Bilirubin	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase	Total Protein (g/dL)	Blood Urea (mg/dL)	Sr. Creatinine (mg/dL)	Sr. CPK	Blood Sugar (mg/dL)
4.6	50	45	96	6	38	1.2	420	111
5.8	40	36	112	6.2	28	0.9	610	92
2.2	52	48	102	5.8	98	3.7	540	128
2.6	46	50	96	5.8	42	1.4	316	70
5	240	232	180	6.2	46	1.5	240	76
5.3	40	36	126	6.4	110	4.1	656	92
4.8	72	64	112	7	30	0.8	180	84
1.8	90	92	140	6.4	84	3.2	245	110
5.1	40	41	84	5.8	28	0.9	360	116
2.1	40	38	176	6.8	26	0.9	450	98
1.8	42	36	152	7	34	1.2	410	76
4.1	40	44	86	7	32	1	520	62
4.6	42	40	92	6.8	28	1.1	634	140
3	40	36	106	5.6	42	1.4	130	96
2	42	38	112	5.8	96	3.4	270	132
2	54	50	86	6	41	2.3	310	121
3.3	198	196	240	6.8	60	1.5	570	126
5.1	40	36	118	6.8	70	1.8	530	96
2.1	42	40	96	7	64	1.7	310	92
1.4	38	45	64	6.6	32	0.9	110	98
3.4	50	56	102	6.4	36	1.1	129	104
2.1	62	64	92	6.8	42	1.7	260	96
1.7	40	36	112	7.2	62	1.6	140	136
3	70	72	122	7	32	1	320	126
2	84	90	142	6.8	94	3.1	190	121
4.1	42	40	102	5.6	32	1	380	96
4.4	38	40	114	6.8	28	1.2	430	92
11.7	117	106	296	6.8	127	7.4	90	84
6.1	42	40	76	7.2	34	1	410	72
3.5	56	63	160	5.8	60	2.6	620	70
4.4	40	41	84	5.9	34	1.2	325	96
3.1	38	32	96	6	32	1	420	122
6.1	40	36	108	7	36	1.2	290	126
6.5	120	126	186	6.8	38	1.6	200	96
3.6	40	36	110	6.4	40	1.8	170	74
1.4	38	41	86	5.9	78	2.4	350	94
3.2	45	41	90	6.2	32	1.1	400	96
2.1	38	36	92	6.1	40	1.2	320	106
3.1	40	51	78	5.6	38	1.1	610	94

5.2	22	18	53	5.1	126	6.2	590	95

Repeat Bilirubin level	Duration of Stay	OUTCOME
1.2	13	Cured
4.1	8	Cured
	4	Expired
0.9	8	Cured
	2	Expired
	3	Expired
2.9	20	Cured
1.1	10	Cured
1.3	8	Cured
1.1	11	Cured
4.9	18	Expired
2.4	8	Cured
2.6	9	Cured
1.2	17	Cured
1.1	7	Cured
1.7	4	Cured
1.9	9	Cured
2.8	10	Cured
	6	Expired
1.4	20	Cured
1.5	12	Cured
1.7	11	Cured
	4	Expired
0.9	9	Cured
1.2	10	Cured
3.3	8	Cured
2.4	17	Cured
6.8	21	Cured
3.1	12	Cured
	3	Expired
0.9	9	Cured
0.8	11	Cured
3.1	9	Cured
2.3	10	Cured
1.7	8	Cured
1.3	9	Cured
	4	Expired
1.1	13	Cured
1.4	16	Cured

3.1	19	Cured